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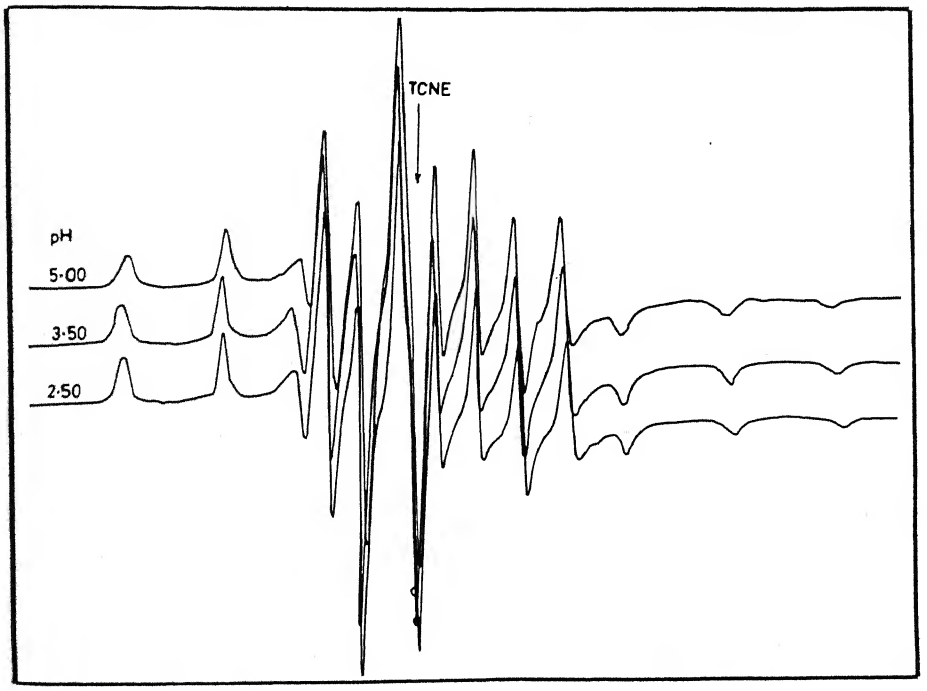

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Studies on the effects of leaf leachates of *Pongamia pinnata* on certain crops and weeds and the soil mycoflora

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Abstract The allelopathic effects of the leachates of the leaves of *Pongamia pinnata* against rice, wheat, *Cassia tora* and *C. occidentalis* were studied. The leachates inhibited the performance of both rice and wheat, but exerted no effect on the weeds. The leachates of *P. pinnata* contained allelochemicals such as vanillic acid, syringic acid, melilotic acid and derivatives of quercetin and kaempferol. The residual phenolics of the soil were more in the case of the weeds. The variety of mycoflora below *Pongamia* were less compared to control.

(Keywords : allelopathy/Pongamia/agroforestry/ mycoflora/residual phenolics)

Pongamia pinnata Pierre (*P. glabra* Vent.), a tropical deciduous tree native to Western ghats (commonly known as 'Karanj'), is a multipurpose tree recommended in agroforestry programmes. This tree is the source of karanj oil, timber and fuel[1]. The leaf extract is antibacterial, juice prescribed for flatulence, dyspepsia, diarrhoea, cough and leprosy. The stem wood is used as timber, fuel and for furniture making. Stem bark finds use against bleeding piles and it's decoction is used against beriberi. The seed has a hypotensive principle and also used in bronchitis, leprous sores and skin diseases. Pongam oil is used in the manufacture of soap and finds use against leucoderma and rheumatism also. The plant is a repository of many polyphenols like furanoflavones, chromenochalcones and isoflavones. The leaf extract is reported to have 3'-methoxypongapin[2], 7- methoxyfuranoflavone, 8- methoxyfuranoflavone[3], kanjone, karanjin[4], *beta*-sitosterol, pinnatin glabrachromene[5] and various other compounds. Though a number of studies on the various aspects of this plant were made, it's allelopathic interactions with other plants were never evaluated. The poor

undergrowth below this tree is remarkable, showing it's ability to suppress the growth of other plants and therefore, this plant may be considered as a potential source of herbicides. Therefore the present study was conducted to find out (1) the allelopathic interaction of the leaves of *P. pinnata* on the common crops *viz.*, rice and wheat, (2) the herbicide potential on two common weeds, *Cassia tora* and *C. occidentalis*, (3) allelochemicals in the leaves of *P. pinnata*, (4) residual phenolics remaining in the soil and (5) the mycoflora in the soil below the tree.

Certified seeds of wheat (*Triticum aestivum* var. Lok1) and rice (*Oryza sativa* var. GR11) were procured from Gujarat State Seeds Corporation, Vadodara, Leachates of the leaves were prepared by keeping 5g of the dried powdered material in 100 ml distilled water (100%) for 48 hr. and filtering. This filtrate is diluted in distilled water to make desired concentrations of 20%, 40%, 60% and 100%. 500g of soil, washed free of organic matter, were irrigated with desired amount of the plant extracts of different concentrations separately. The soil samples containing distilled water was maintained as the control. Three replicates were maintained for each treatment. Seeds of the crops and weeds were surface sterlised in 0.1 % mercuric chloride for 15 min. and washed thoroughly in distilled water. Ten seeds were sown in each replicate separately. They were incubated at room temperature for 14 days. Results were taken on the 15th day after incubation. The parameters looked for were, percentage of seed germination, root length, shoot length and total dry weight of the seedlings. The experimental data were analysed by ANOVA and multiple range test using least significance difference (LSD) procedure.

Screening of the allelochemicals in the leaves were done by extracting 50g of dried, powdered leaves with methanol in Soxhlet's apparatus for 48 hours. Aglycones obtained by acid hydrolysis of the crude extract were separated by ethyl acetate. The ethyl acetate fraction was analysed for allelochemicals like phenolic acids and flavonoids using standard methods. For identifying phenolic acids the method of Ibrahim and Towers[6], in which diagnostic reagents like diazotised *p*-nitroaniline and sulphanilic acid in two-dimensional PC were used. For flavonoid identification, UV spectral analysis[7] involving the bathochromic and hypsochromic shifts with reagents such as $AlCl_3$, $AlCl_3$/HCl, NaOAc, NaOAc/ H_3BO_3 and NaOMe were carried out. The identities of both phenolic acids and flavonoids were confirmed by co-chromatography with authentic samples.

Estimation of residual phenolics in the sand samples treated with leaf leachates was done by Folin-Ciocalteau's method[8]. One kilogram of sterilised sand

irrigated with 200 mL of leachates of the leaves (10g in 200 mL distilled water) was maintained as control. Other treatments included sand with the same amount of leachate (200 mL) in equal concentration to which five surface sterilised seeds of each crop and weed were sown separately. Among various treatments, different controls were maintained without adding the leaf leachates, but the seeds alone. Three replicates were maintained in each treatment and controls. These were incubated at room temperature for 7 days, after which the phenolics in the soil were extracted in distilled water [9,10]. The filtrates were analysed for total residual phenolics with gallotannic acid as the reference compound.

Study of the mycoflora in the soil below the tree was conducted in the month of November/December when the leaf fall was maximum. 10g of soil samples were collected at a depth of 7 cm. from the ground level at various distances from the tree. Soil samples collected from a barren land served as the control sample. The samples were passed through serial dilutions in distilled water. One milliliter of the final dilution solution was inoculated on petriplate containing 20mL of Potato Dextrose Agar medium. Three replicates were maintained which were incubated at room temperature for 5-7 days. The fungi obtained were pure-cultured on slants containing PDA medium. They were identified and confirmed by Agharkar Research Institute, Pune.

Leachates of *P.pinnata* leaves exerted highly significant inhibitory effect on the germination, dry weight and root length of rice and wheat whereas no significant effect was observed on the shoot length (Table 1). In the case of weeds there was no significant inhibitory effect on any of the parameters studies.

The leachates of *P.pinnata* revealed the presence of potent phenolic allelochemicals like vanillic acid (purple with diazo-*p*-nitroaniline and orange with sulphanilic acid; Rf 0.43 and 0.55), syringic acid (blue/red; Rf 0.43 and 0.45) and melilotic acid (purple/yellow; Rf 0.63 and 0.60). The flavonoids identified were 3', 4'-dimethoxy quercetin ($\frac{\lambda_{max}}{MeOH}$ - 255 nm, 364 nm, + $AlCl_3$ – 430 nm, + $AlCl_3$/HCl–425 nm, + NaOAc – 265 nm, + NaOAc/H_3BO_3 – 390 nm, + NaOMe – 400 nm dec); 4' methoxy quercetin ($\frac{\lambda_{max}}{MeOH}$ - 255 nm, 368 nm, + $AlCl_3$ 438 nm, + $AlCl_3$/HCl–425 nm, + NaOAc – 265 nm, + NaOAc/H_3BO_3 – 390 nm, + NaOMe – 420 nm dec) and kaempferol ($\frac{\lambda_{max}}{MeOH}$ - 267 nm, 367 nm, + $AlCl_3$-425 nm, + $AlCl_3$/HCl–425 nm, + NaOAc – 278 nm, + NaOAc/H_3BO_3 – 268 nm, + NaOMe – 416 nm dec).

Table 1 : Effect of leaf leachate of *P.pinnata* on the germination and seedling growth of the test plants.

Test plants	Dilution level(%)	Germination Percentage		Dry weight (g)		Shoot length (cm.)		Root length (cm.)	
		Mean (± SD)*	% increase (+)/decrease (–)#	Mean (± SD)*	% increase (+)/decrease (–)#	Mean (± SD)*	% increase (+)/decrease (–)#	Mean (± SD)*	% increase (+)/decrease (–)#
O. Sativa	Control	8.3333 (2.1213)	-	0.0752 (0.0192)	-	6.3987 (1.7296)	-	7.5160 (2.7663)	-
	20	8.5556 (2.1279)	+ 2.67	0.0708 (0.0118)	- 5.85	6.4390 (1.6691)	+ 0.63	7.2455 (3.38998)	- 3.59
	40	8.2222 (2.2791)	- 1.33	0.0653 (0.0221)	- 13.16	6.1000 (2.6872)	- 4.67	3.6149 (2.7531)	- 51.90$
	60	6.7778 (2.1667)	- 18.67	0.0526 (0.0229)	- 30.05$	6.0246 (2.0832)	- 5.85	1.8541 (2.0342)	- 75.33$
	100	3.0000 (1.5811)	- 63.99$	0.0277 (0.0174)	- 63.16$	6.2741 (1.3592)	- 1.95	0.9296 (1.4712)	- 87.63$
T. aestivum	Control	4.6667 (1.7233)	-	0.0491 (0.0290)	-	60446 (2.8166)	-	1.2500 (0.6525)	-
	20	2.6667 (1.8257)	- 42.86$	0.0283 (0.0197)	- 42.36$	6.0219 (2.2668)	- 0.38	0.8563 (0.3860)	- 31.49$
	40	3.1667 (1.8505)	- 32.14$	0.0339 (0.0244)	- 30.96	4.9395 (2.2294)	- 18.28	0.6895 (0.4184)	- 44.84$
	60	1.5000 (1.1677)	- 67.86$	0.0158 (0.0120)	- 67.82$	5.0167 (2.3809)	- 17.01	(0.6944) (0.5557)	- 44.45$
	100	1.4147 (1.1645)	- 69.69$	0.0152 (0.0134)	- 69.04$	4.7353 (1.9704)	- 21.66	0.4882 (0.3120)	- 60.94$
C. tora	Control	3.8889 (2.8916)	-	0.0276 (0.0215)	-	10.1171 (2.6656)	-	6.5400 (2.6952)	-
	20	3.3333 (2.6926)	- 14.29	0.0245 (0.0190)	- 11.23	11.5433 (1.8091)	+ 14.09	7.5967 (2.6551)	+ 16.16$
	40	2.8889 (2.7588)	- 25.71	0.0200 (0.0200)	- 27.54	10.5269 (2.8572)	+ 4.05	5.7846 (2.3682)	- 11.55
	60	4.1111 (3.5862)	+ 5.71	0.0270 (0.0254)	- 2.17	10.3703 (1.6561)	+ 2.50	5.3514 (2.3062)	- 18.17
	100	5.1111 (2.6194)	+ 31.43	0.0412 (0.0255)	+ 49.28	9.9870 (2.8427)	- 1.29	6.8174 (2.9157)	+ 4.24
C. occidentialis	Control	3.6667 (3.1225)	-	0.0211 (0.0182)	-	8.0333 (1.9843)	-	4.3121 (2.4251)	-
	20	2.4444 (1.3333)	- 33.34	0.0152 (0.0092)	- 27.96	8.1500 (1.7331)	+ 1.45	5.4818 (2.2673)	+ 27.13
	40	2.6667 (1.5811)	- 27.27	0.0185 (0.0112)	- 12.32	7.8250 (2.1334)	- 2.59	5.9458 (2.7872)	+ 37.89
	60	4.5556 (1.8782)	+ 24.24	0.279 (0.0143)	+ 32.23	8.0244 (1.8404)	- 0.11	5.4780 (2.5391)	+ 27.04
	100	2.6667 (1.1180)	- 27.27	0.0143 (0.0080)	- 32.23	7.1208 (1.9045)	- 11.36	5.3958 (2.6290)	+ 25.13

*Average of 45 observations # Average percentage of increase (+) or decrease (-) when compared to respective control $ Values are significantly different at $p < 0.05$

Table 2 – Tatal residual phenolics of *P. pinnata* leaf leachate in soil samples

Treatments	Total phenolics (μ g/100g)
Control	2.0
Extract + rice seeds	3.3
rice seeds	3.2
Extract + wheat seeds	0.19
wheat seeds	2.0
Extract + *C. tora* seeds	36.8
C. tora seeds	56.7
Extract + *C. occidentalis* seeds	30.0
C. occidentalis seeds	22.7

In all the treatments, residual phenolic allelochemicals were found to be in comparatively higher concentration than the control. The treatments with weed species contained much higher concentration of residual phenolics than those of the crops. It was also observed that the soil samples having weed species alone (without *Pongamia* leaf leachate) contained considerable amounts of phenolics (Table 2).

Most of the fungi isolated from under *P.pinnata* tree belonged to the genera *Aspergillus* and *Mucor*, all being saprophytic in habit, whereas those of control soil were many varieties of fungi belonging to both parasitic and saprophytic species. They were *Curvularia pallescens*, *Septoria* sp., *Phoma glomerata*, *Myrothecium roridum*, *Aspergillus niger*, *A. flavus* and *Mucor racemosus*.

The negative allelopathic potential of *P.pinnata* leaves is amply evident from the present study, in that leachates of this tree inhibited the percentage of germination, growth of root any dry weight of seedlings of both the crops studied. The presence of known allelochemicals such as vanillic acid[11,12,13], syringic acid[14,15] and kaempferol[16] in the leaves of *P.pinnata* supports the effects observed in the experiment. However, it does not have any action on the two weeds studied i.e. *Cassia tora* and *C. occidentalis*. This

necessitates further study to find out similar interactions of *P.pinnata* on other crops and weeds after which only this tree be recommended in agroforestry programmes. The study on the residual phenolics throws light on the probable mechanism which was active in the treatments. Higher amounts of phenols secreted by germinating seeds or seedlings of both the weeds i.e. *Cassia tora* and *C. occidentalis* is noteworthy. It may be inferred that the large amounts of these compounds possessed by the weeds might have neutralised the allelochemical phenolics of *P.pinnata* from causing any change in the perfor-mance of the seeds of the weeds. The very less amount of phenolics possessed by both the crops might have been the reason for the inhibitory action of *P.pinnata* on crops. Therefore the proposed herbicidal potential of *P.pinnata* against these weeds does not get any support from the present investigation. The absence of the large variety of fungi as also of the pathogenic species in the soil below *P.pinnata* may indicate it's antimicrobial activity of the chemicals released from the plant.

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Regulation of siderophore production by iron (Fe III) in certain mucoraceous fungi

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Abstract Eleven fungi belonging to Mucorales that produced carboxylate siderophores, varied widely in their response to ambient iron (Fe III) stress. *Absidia ramosa* did not produce siderophores even at 0.5 µM Fe III, while some *Rhizopus* sp. produced even at highest test concentration of 18-21 µM. The siderophore production, however, decreased with increase in Fe III concentration.

(**Keywords** : siderophore/iron/mucorales/rhizoferrin)

Siderophore (sid=iron, phores=bearers) are low molecular weight iron-chelating compounds produced by microorganisms as scavenging agents to combat low iron stress[1,2]. At a critical threshold value of Fe (III), the synthesis stops. For most of the fungi examined it is 5 µM[3], while for bacteria it varies between 0.5 µM–25 µM[4] Fe(III). It is interesting that no element other than iron involves a mechanism similar to siderophore production in microorganisms.

Siderophore biosynthesis is known in much detail for enteric bacteria, especially *Escherichia coli*[3], but only little information is available for fungi. This study aims at examining the threshold concentration of Fe(III) that represses siderophore production in certain mucoraceous fungi.

Eleven fungi belonging to Mucorales were isolated from soils from and around Bhavnagar. The organisms were maintained on potato dextrose agar slants (PDA) and stored at 4 °C. In preliminary experiments all these fungi produced carboxylate siderophores in CAS (chrome azurol sulfonate) assay[5], which is based on the observation that when a strong chelator removes iron from CAS/Iron/ detergent complex, the dye colour changes from blue to orange. In electrophoretic mobility test[5] these siderophores were mostly basic. In the present study the eleven test fungi were grown on Modified M9 medium[6] which was rendered iron-free by using 8 hydroxy-quinoline dissolved in chloroform as suggested by Messenger and Ratledge[7]. The medium was supplemented with varying test concentrations of Fe (III) (0.5 to 27 µM) with a view to observe the threshold concentration that repressed siderophore production. The fungi were grown at 30 °C (except *Mucor mucedo* which grew at 25 °C) and 15 days old culture-filtrates made cell-free by filtration through Whatman 42 filter paper, were examined for presence for siderophores by CAS assay[5]. The amount

Table 1 – Threshold concentration of $FeCl_3$– for siderophore induction/repression by fungi (+ indicates induction and - repression; the values indicate amount of siderophore, X 10^{-4} g/mL).

Test Fungi	0.5µM	1.0µM	3µM	6µM	9µM	12µM	15µM	18µM	21µM	24µM	27µM
Absidia ramosa	-	-	-	-	-	-	-	-	-	-	-
Absidia sp.	+	+	-	-	-	-	-	-	-	-	-
	9.4	3.5									
Cunninghamella echinulata	+	+	+	+	+	-	-	-	-	-	-
	21.2	10.3	8.4	3.5	6.5						
C. elegans	+	+	-	-	-	-	-	-	-	-	-
	7.9	4.4									
Mucor mucedo	+	+	+	+	-	-	-	-	-	-	-
	10.3	9.4	8.4	7.4							
Rhizopus microsporus	+	+	+	+	+	+	+	+	-	-	-
	27.1	26.6	21.2	19.7	18.7	1.3	0.7	8.9	-	-	-
R. nigricans	+	+	+	+	+	+	+	+	+	-	-
	11.8	8.9	8.4	7.4	5.9	4.9	4.4	4.2	1.7		
R. oryzae	+	+	+	+	+	+	+	+	-	-	-
	2.8	2.4	2.3	23.2	21.2	19.2	13.8	9.9			
R. rhizopodiformis	+	+	+	+	+	+	+	-	-	-	-
	23.1	10.3	4.9	4.4	3.5	3	4.6				
Syncephalastrum racemosum	+	+	+	+	+	-	-	-	-	-	-
	17.2	13.3	9.9	11.1	4.4						
Syncephalastrum sp.	+	+	+	+	+	+	+	+	-	-	
	17.7	12.3	11.1	4.4	3.9	3	2	1			

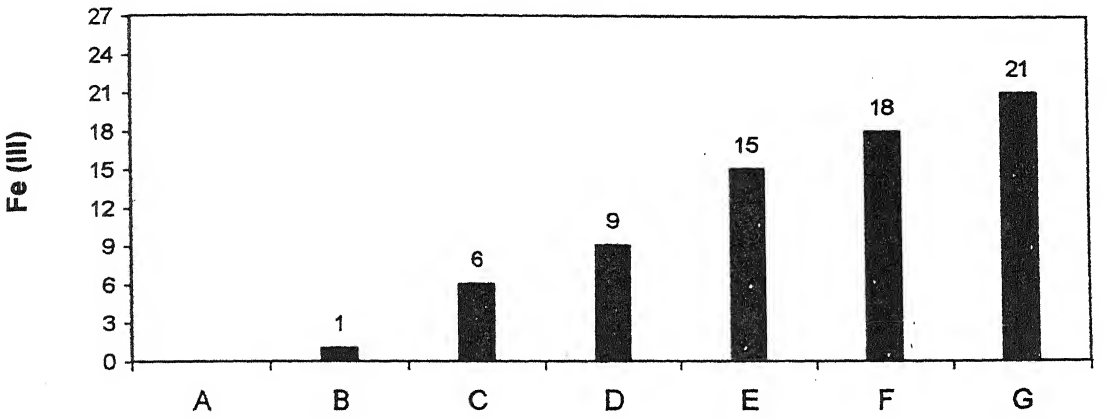

Fig. 1-Repression of siderophore production by fungi at iron levels 0.5-27μM/L

A : *Absidia ramosa* B : *Absidia* sp., *Cunnighamella elelgans* C : *Mucor mucedo*,D : *C. echinulata, Syncephalastrum racemosum*, E : *Rhizopus rhizopodiformis*, F : *R, microsporus, R. oryzae, Syncephalastrum sp.*, G : *R. nigricans*

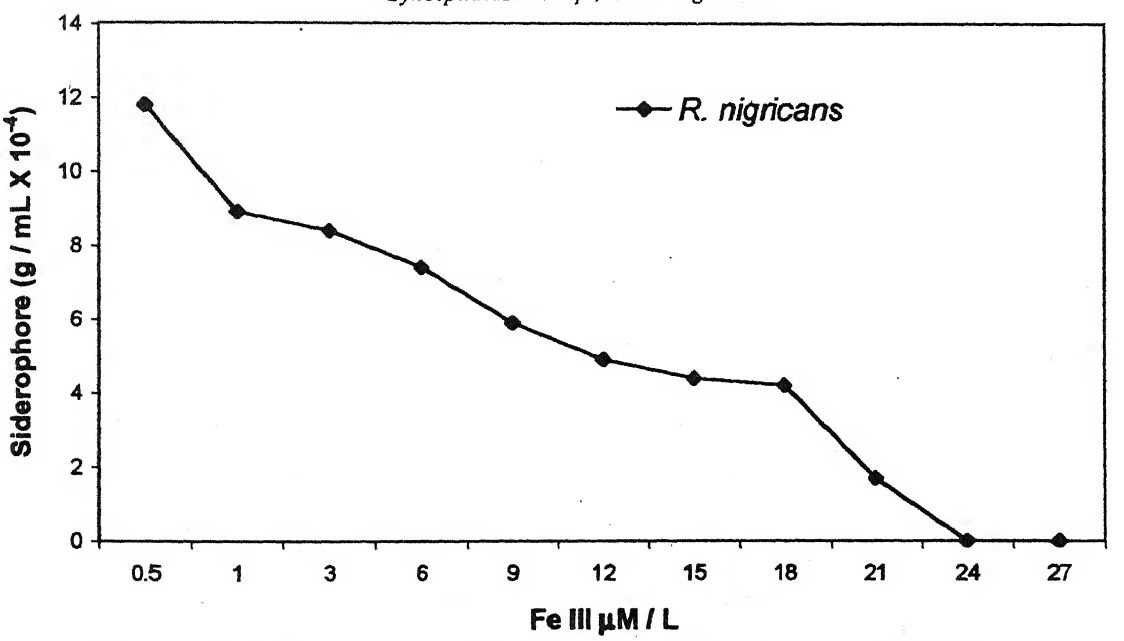

Fig. 2 - Showing decrease in siderophore production (g/ML) with rise in iron (Fe III) concentration (0.5 μM/L to 24 μM/L) of *R. nigricans*

of siderophore produced was measured from the standard curve for the CAS assay by analyzing the absorbance (630 nm) of each standard solution (rhizoferrin-standard carboxylate siderophore) divided by the absorbance (630 nm) of the reference solution (A/A_{ref}) as a function of siderophore concentrations in the range of 20–100 μM, that yielded a linear relationship[8].

The results (Table 1) showed great variation in the threshold iron concentration that repressed siderophore production by the different test fungi. At the one extreme was *Absidia ramosa*, which was repressed at the lowest iron concentration of 0.5 μM, while at the other end was *Rhizopus nigricans* in which the biosynthesis continued up to 21 μM Fe (III). Rest of the organisms produced siderophores between concentrations 0.5-21 μM. Fig. 1 shows the range of concentration at which the siderophore biosynthesis occurred in the different test fungi.

According to the repressibility of siderophore synthesis the fungi can be arranged in the following sequence. *A. ramosa*, 0.05 μM > *Absidia* sp. And *C. elegans*, 1.0 μM > *M. mucedo*, 6 μM > *C. echinulata* and *S. racemosum*, 9 μM > *R. rhizopodiformis*, 15 μM > *R. microsporus*, *R. oryzae* and *Syncephalastrum* sp., 18 μM > *R. nigricans*, 21 μM. This suggests that *A. ramosa* is most sensitive to iron-repression for siderophore production, while *R. nigricans*, which continued siderophore production even at high concentration of 21 μM, is least amenable to iron-repression. The results also suggest that siderophore production (g/ml) gradually decreases with increased iron concentration as explained for *R. nigricans* in Fig. 2.

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Botanical drug for therapy against fungal infections in human beings

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Abstract Botanical drug for fungal infections is a supplement to synthetic drug. Thus is formulated from the essential oil of *Eucalyptus dalrympleana*. The oil of *E. dalrympleana* was most potent antifungal agent, which completely inhibited the mycelial growth of test pathogens, *Epidermophyton floccosum, Microsporum gypseum* and *Trichophyton rubrum*. The minimum inhibitory concentration (MIC) of the oil was found to be 0.3 µl ml^{-1} for aforesaid pathogens. The oil (0.3 µl ml^{-1}) also exhibited potency against heavy doses of inocolum (30 mycelial discs each of 5 mm diameter). Moreover, this oil preparation did not adversely affect mammalian skin upto 5% level. Further, oil was used in the form of ointment for topical testing on patients, attending out patient department (OPD) of M.L.N. Medical College, Allahabad, At the end of medication, 50.0% of patients recovered completely and 40.0% showed significant improvement. No KOH negative cases of relapse were observed when patients were reexamined after two months following treatment. It indicates the absence of relapse. The ointment was found to be cost effective (INR 1.0/g), has long shelf life (48-months) and devoid of any adverse effects. Thus, the oil preparation could be used as a potential antifungal agent after the successful completion of multicentre clinical trial.

(**Key words** : antifungal drug/fungal infection/ natural product)

Dermatophytoses is the disease caused by a group of fungi known as dermatophytes[1]. These are also called as ringworm or tinea and affect superficial keratinized tissue of the skin of animals and human beings. Clinical surveys carried out in India show that ringworm is one of the commonest dermatomycoses caused by different species of *Trichophyton, Microsporum* and *Epidermophyton*[1]. This disease is predominant in tropical and subtropical countries due to heat and humidity and poses a therapeutic problem[2,3]. Thus, meaningful searches of new therapy with better and cheaper substitutes-based on plant resources are a natural choice. Recently, some plant products have been shown to be effective source of chemotherapeutic agents without undesirable side effects[4-7]. The present paper reports the results of *in vitro* studies on the antifungal activity of *E. dalrympleana* oil against dermatophytes and clinical efficacy of the formulated product.

The essential oil was extracted from the leaves of *E. dalrympleana* by hydro-

distillation using Clevenger's apparatus[8]. A clear light-yellow-coloured oily layer was separated and dried with anhydrous sodium sulphate.

The minimum inhibitory concentration (MIC) of the oil against dermatophytes was determined following the poisoned food technique (PFT) of Grover and Moore[9] with slight modification[6]. The requisite quantity of the oil sample was mixed in acetone (2%) and added to pre-sterilized Sabouraud dextrose agar (SDA) medium (pH-5.6). In control sets, sterilized water (in place of oil) and acetone were used. Mycelial discs of 5 mm diameter, cut out from the periphery of 7-day old cultures of the test dermatophytes. Pathogens were aseptically inoculated upside down and plates were incubated as 27±1°C for seven days. Percentage of mycelial growth inhibition (MGI) was calculated as follows :

$$MGI\ (\%) = (dc-dt)\ 100/dc$$

dc = fungal colony diameter in control sets

dt = fungal colony diameter in treatment sets

The nature of antifungal activity, fungistatic/fungicidal was determined by a method of Garber and Houston[10]. The inhibited fungal discs at MICs were reinoculated on SDA medium. Fungal growth on seventh day indicated fungistatic nature while absence of fungal growth denoted fungicidal action of the oil.

The effect of inoculum density (increased progressively up to 30 discs in multiple of 5 and each of 5 mm diameter) of the test pathogens on antifungal activity of the oil was determined following the procedure outlined by Dikshit and Dixit[11]. Effect of temperature and expiry of toxicity during storage of the oil was evaluated according to Shahi *et al*[7]. Five lots of oil were kept in small vials, each containing 5 ml of oil; these were exposed to 40, 60, 80°C in an incubator for 60 minutes; residual activity was assayed by poisoned food technique[9]. Expiry of toxicity of the oil under storage was determined at room temperature.

Sample was withdrawn at an interval of 60 days up to 48 months and tested following poisoned food technique[9]. All the experiments were repeated twice and each contained five replicates; the data represent mean values.

To determine the maximum tolerable concentration (MTC) and long term toxicity for irritant activity, if any, the oil for topical application on human skin, the procedure of Shahi *et al*[7] was adopted. People of different sex in the age group of 10 to 30 years were selected randomly and a group of 30 individuals of each sex was constituted. Circular areas of 5 cm^2 on upper hairy

and lower glabrous surface of palms and 3 cm^2 of neck region of each individuals were first washed with distilled water followed by 70 percent ethyl alcohol, and allowed to dry for five minutes. Five drops of the graded concentrations of testing solution were applied to each individual separately for three weeks. The volunteers were not allowed to wash the applied areas. The qualitative observations were recorded after an interval of 24 hr, up to three weeks.

Clinical response to the ointment : An experiment was designed to test the activity of oil based ointment (1%, w/v) on patients for control of superficial fungal infections. The ointment was applied twice a day for 4 weeks. The patients were not allowed to take any other systemic or topical therapy during the course of study.

Patients selection : Fifty patients, of either sex within the age group, 8 to 40 years and suffering from tinea corporis, tinea cruris or tinea pedis were randomly selected for the study. The diagnosis was confirmed mycologycally by KOH examination. To minimize inconvenience to the patients additional 50 patients were selected as control.

Methods : Patients were examined just before the therapy was initiated and at the end of each week for 4 weeks of treatment. When fungal disease affected several body areas, all affected areas were treated, but only one was selected and designated as the reference lesion. At each visit of the patient, the same reference lesion was scraped for fungal culture to identify the organism and for demonstration of the presence of hyphae by microscopic examination with 10% potassium hydroxide (KOH). Signs and symptoms of diseases i.e., erythema, oedema, scaling, maceration, vesiculation, pustulation, crusting and itching were graded as absent (–), mild (+), moderate (++), or severe (+++) and noted at every visit and scores were added. Less than 25% improvement was considered no improvement, 25–50% as mild, 50–75% as moderate, >75% as significant and complete. Any adverse systemic or local reaction was noted at each visit. Satisfactory response with KOH negative cases after 4^{th} week was re-examined after two months to find out the relapse rate, if any.

Results and Discussion : The oil from *E. dalrampleana* showed a broad range of antifungal activity at 0.3 µl ml^{-1} against *Epidermophyton floccosum, Microsporum audouini, M. canis, M. gypseum, M. nanum, T. mentagrophytes, T. rubrum, T. tonsurance* and *T. violaceum.* (Table 1). The oil inhibited heavy doses of inoculum (30 mycelial discs each of 5 mm in diameter) at its respective fungicidal concentrations. The fungicidal activity of the oil

persisted up to 80°C and it didn't expire up to 48 months of storage. When tested for its irritant activity and long term toxicity on human skin, the oil did not show any irritation or adverse effect at 5% levels up to 3 weeks. Formulation was prepared using 1% oil in the ointment. After topical application of the ointment, improvements were observed in the treated patients right from the first week; 40.0% showed moderate improvement, 40.0% mild improvement while 20.0% showed no improvement. After second week, 25.0 and 35.0% showed mild improvement while 20.0% no improvement. After second week, 25.0 and 35.0% patients exhibited significant and moderate improvement respectively. However, at the end of medication (i.e. after 4th week), 50.0% of the patients were classed as completely clear and 40.0% as significant improvement while 10.0% were placed in the category of moderate improvement (Table 2). Majority of the control patients showed no improvement. None of them showed more than a mild improvement.

Table 1–Minimum inhibitory concentrations of the oil of *Eucalyptus dalrympleana* against test pathogens.

Concentration	Mycelial growth inhibition (MGI %)								
($\mu l\ ml^{-1}$)	*Ef*	*Ma*	*Mc*	*Mn*	*Mg*	*Tm*	*Tr*	*Tt*	*Tv*
1.0	100^c	100^c	100^c	100^c	100^c	100^c	100^c	100^c	100^c
0.9	100^c	100^c	100^c	100^c	100^c	100^c	100^c	100^c	100^s
0.8	100^s	100^c	100^c	100^s	100^c	100^s	100^c	100^s	100^s
0.7	100^s	100^s	100^c	100^s	100^s	100^s	100^s	100^s	100^s
0.6	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s
0.5	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s
0.4	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s
0.3	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s
0.2	86.3	87.1	76.2	75.0	87.9	78.3	69.9	77.3	86.3
0.1	40.1	33.3	46.1	32.2	31.1	43.2	20.1	30.2	39.2

Ef = Epidermophyton floccosum, Ma = Microsporum audouinii, Mc = M. canis, Mn = M. nanum, Mg = M. gypseum, Tm = Trichophyton mentagrophytes, Tr = T. rubrum, Tt = T. tonsurance, Tv= T. violaceum, s = fungistatic, c = fungicidal.

During cost-benefit analysis[7] of the herbal product compared with synthetic antifungal drugs, the ointment was found to be more effective and less

expensive (Rs. 1.0/g). It has long shelf life (48 month) and has no adverse effects.

Table 2–Fifty patients clinical response to oil preparation (1% ointment).

Parameter	Patients showing clinical response for 4 weeks							
	1		2		3		4	
	T	C	T	C	T	C	T	C
No improvement	20	100	10	80	00	70	00	65
Mild improvement	40	00	30	20	10	30	10	35
Moderate improvement	40	00	35	00	25	00	10	00
Significant improvement	00	00	25	00	30	00	40	00
Complete clear	00	00	00	00	35	00	50	00
T test paried difference								
SD	48.9		41.3		44.8		47.5	
SE of Mean	21.9		18.5		20.0		21.2	

T, treatment; C, control

Based on *in vitro* observation, the oil preparation (ointment) was tested *in vivo* to confirm its efficacy as herbal therapeutant for the control of dermatophytoses.

According to Polak[12] and Pierard *et al*[13], the evaluation of antifungal comounds by *in vitro* tests and experiments on animals cannot predict clinical efficacy with certainty. Hence the clical trials conducted by Shahi[6], and Shahi *et al.*[7] were followed in the present study.

No drug is known to give uniform result with every patient. In general, it depends on the response of the patient's immune system, which varies with the age of the individual. Therefore, if any natural product responds favourably in 50% cases, can be used as an alternative to synthetic drug, since it is harmless. Besides, prolonged therapy may give better results. Thus, due to strong fungicidal activity, long shelf life, absence of any adverse effects and better clinical response, the oil of *E. dalrympleana* can be used as herbal therapeutant for dematophytoses. The commercial viability of the herbal chemotherapy can be determined after successful multicentre clinical trials.

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Taenionella lignicola sp. nov. from India

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Abstract : An undescribed fungus collected on decaying wood was critically examined and identified as *Taenionella* (Karst.) Hughes. On critical observation the fungus was found to differ from the earlier recorded species in the size and shape of the conidia, hence described as *Taenionella lignicola* sp. nov.

(Keywords : hyphomycete/conidia/wood.)

There are 25 species reported in literature (Hawksworth *et al.*, 1991). In the present study, a fungus was assigned to *Taenionella* Hughes but differing from all the known species, conidial morphology and measurements, hence described as new species, *Taenionella lignicola.*

Taenionella lignicola C. Manoharachary, N. Krishna Rao & D. K. Agarwal sp. nov. (Fig. 1).

Coloniae effusae, fusca vel furca; mecelia immersum, hyphae ramosae, septatae, bruneae 1.5-2.0 µm crassus hypha; Conidiophora semi macronematosa, mononematosa, rectus vel flexuosus, laevis, tuni tunicata, perbrevis, fere micronematosa; Cellulae conidiognae mono vel polyblasticae, integratae, terminalis et intercalaris, determinatae, cylindrica 9.0-10.0 x 7.0-8.0 µm. Conidia solitaria, sicca, acrogenosa, non ramosa vel ramosa, rectus vel flexuosus, rotundata et apice, truncata et basi, laevis, crassitunicatus, 2-28 septata, crassa septata, 30.0-125.0 µm longus, 6.5-7.2 µm latae.

Taenionella lignicola C. Manoharachary, N. Krishna Rao & Agarwal sp. nov. (Fig. 1).

Coloniae effuse, deep brown to blackish brown; mycelium immersed; hyphae branched, septate, smooth brown, 1.5-2.0 µm thick hyphae; conidiophores semi macronematous, mono-nematous; conidiogenous cells mono or polyblastic, integrated, terminal also intercalary, determinate, cylindrical, 9.0-10.0 x 7.0-8.0 µm. Conidia solitary, dry, acrogenous, simple or in branched chains, straight or flexuous, rounded at the apex truncate at the base, thick walled, 2-28 septate, thick, 30.0 – 125.0 long, 6.5 – 7.2 µm wide.

Collected on decaying wood, Kalaeswaram forest, Karimnagar District Andhra Pradesh, India, 5-01-1984, Coll. N. K. Rao. OUMH 117 (Holotype IMI,-296861).

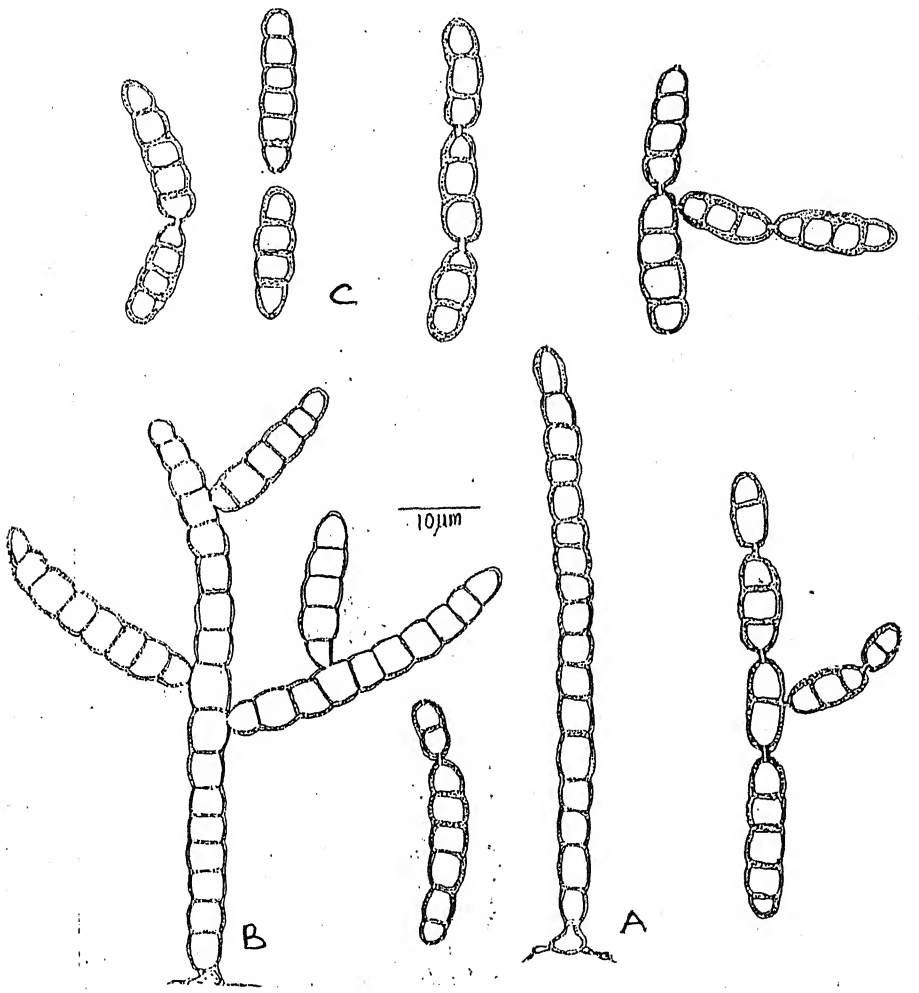

Fig. 1– *Taenionella lignicola* sp. nov. A. Conidiogenous cells bearing mononematous conidiophore chains, B. Conidiophores with branched chians of condia, C. Conidia

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Solution equilibrium study of Cu(II), Ni(II) and Zn(II) complexes with salicylalanine and imidazole

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Abstract The stability constants of the mixed-ligand complexes formed by Cu(II), Ni(II) and Zn(II) with salicylalanine and imidazole has been investigated in aqueous solution by means of potentiometry and spectrophotometry. The stability constants of the binary, ternary and quaternary complexes have been determined at 25±1°C in 0.1 M $NaClO_4$. The experimental data have been obtained for the following species (L = salicylalanine and imH = imidazole) : $[M_2(L)_2(im)]^{2-}$, $[M^1M^2(L)_2(im)]^{2-}$ and $[[M^1M^2(L)_2(im\text{-}H)]^{3-}$. N_1H deprotonation of bidentate coordinated bridging imidazole ligand in the binuclear complexes at pH > 7 is evident from spectral measurements. Stability constants of binary $[ML]^+$, $M(im)^{2+}$ and ternary $[M(L)(im)]^+$ complexes follow the Irving-Williams order.

(Keywords : solution equlibrium/Cu(II)/Ni(II)/Zn(II)/salicylalanine/imidazole)

The aqueous coordination chemistry of imidazolate bridged complexes has been the subject of many investigations for inorganic chemists. Imidazole as a ligand plays important role in biological systems, since imidazole moiety of the histidine residue in a large number of metalloproteins all or part of the binding sites of many transsition metal ions[1-3]. Its conjugate base, imidazolate anion is known to act as a bridging ligand in certain metallo enzymes between Cu^{2+} and Zn^{2+} in bovine erythrocyte superoxide dismutase (BESOD)[4-7]. The literature survey reveals very scarce data[8,9] on the aqueous coordination chemistry of imidazolate bridged complexes. Recently our school has reported[10-12] solution equilibrium study of imidazolate bridged complexes with Cu^{2+}, Ni^{2+} and Zn^{2+}. The study is now extended by taking salicylalanine (salala) and hence metal ions (Cu^{2+}, Ni^{2+} and Zn^{2+}) and imidazole as bridging ligand in aqueous medium at 25±1°C and I=0.1 M$NaClO_4$.

Reagents and solutions : All solutions were prepared by using glass double-distilled water. A CO_2 free NaOH solutions was prepared under an atmosphere of nitrogen. It was kept in polyethylene bottle with sodalime guard tubes. All solutions were made upto an ionic strength of 0.1 M $NaClO_4$.

Protonation constants of the ligands and stability constants of the copper(II) complexes were determined in a 0.1 M

$NaClO_4$ at 25°C by using a Systronics pH-meter (335-pH meter). The spectra were recorded on a Systronics UV-VIS Spectrophotometer UV-117 with 1 cm quartz cell in aqueous solution. The total volume of the solution was 50 ml. The solution in the vessel was stirred continuously by using magnetic stirrer. The temperature was maintained using a Yorko Thermostat.

For the quaternary systems, following sets of solution were prepared in (total volume 50 ml) for titrations : (1) 0.03 M perchloric acid + 0.1M $NaClO_4$ (2) 0.03 M perchloric acid + 0.003 M salala + 0.1M $NaClO_4$, (3) 0.03 M perchloric acid + 0.003 M salala + 0.003 M metal(II) perchlorate + 0.1M $NaClO_4$ (4) 0.03 M perchloric acid + 0.003 M imH + 0.1 M $NaClO_4$, (5) 0.03 M perchloric acid + 0.003 M metal(II) perchlorate + 0.003 M imH + 0.1 M $NaClO_4$, (6) 0.03 M perchloric acid + 0.003 M metal(II) perchlorate + 0.003 M salala + 0.003 M imH + 0.1 M $NaClO_4$, (7) 0.03 M perchloric acid + 0.006 metal(II) perchlorate + 0.006 M salala + 0.003 M imH + 0.1 M $NaClO_4$, (8) 0.03 M perchloric acid + 0.003 M metal perchlorate (M^1) + 0.003 metal perchlorate (M^2) + 0.006 M salala + 0.003 M imH + 0.1 M $NaClO_4$.

Each one of the above samples was titrated against 1.0 M sodium hydroxide.

Ionisation constants : Salicylalanine has three possible metal coordination sites, the carboxylate, amide and phenolate oxygen atoms or if the amide group is deprotonated, the deprotonated nitrogen. In a potentiometric titration with 0.1M NaOH two moles of base are consumed per mole of Salala. The pK values were calculated and presented in Table1.

Table 1–Stability constants of ligands at 25±1°C and I=0.1 M $NaClO_4$ (standard deviations are ±0.02 in log units).

H^+ Complexes	log β_{00rst}
LH_2^-	11.70
LH^{2-}	8.26
imH^+	7.10

Table 2–Hydrolytic constants (log β_{p000t}) of M^{2+} (aq.) ions.

Complexes	Ni(II)	Cu(II)	Zn(II)
$M(OH)^{+}$	-8.10	-6.29	-7.89
$M(OH)_2$	-16.87	-13.10	14.92

Table 3–Stability constants (log β_{porst}) of M^{2+} complexes (binary complexes) at 25±1^{0}C and I=0.1 M $NaClO_4$ (standard deviations are ± 0.02 in log units).

Complexes	Ni(II)	Cu(II)	Zn(II)
$[ML]^{-}$	2.89	5.38	3.14
$[ML(OH)]^{2-}$	-1.40	1.00	-2.70
$M(im)^{2+}$	3.65	4.31	2.53

Table 4–Stability constants (log β_{porst}) of M^{2+} ternary complexes at 25±1^{0}C and I = 0.1 M $NaClO_4$ (standard deviations are ± 0.02 in log units)

Complexes	Ni(II)	Cu(II)	Zn(II)
$[MLim]^{-}$	6.32	8.86	6.01
$[M_2(L)_2(im)]^{2-}$	16.61	19.50	15.76
$[M_2(L)_2(im\text{-}H)]^{3-}$	9.81	13.79	8.39
	(6.80)*	(5.89)*	(7.37)*

*pK values of homo-binuclear complexes are shown in parenthesis

Table 5–Stability constants (log β_{pqrst}) pg M^{2+} quaternary complexes at 25±1^{0}C and I = 0.1 M $NaClO_4$ (standard deviations are ± 0.02 in log units)

Complexes	Cu-Zn	Cu-Ni	Zn-Ni
$[M^1M^2(L)_2(im)]^{2-}$	19.05	20.35	17.73
$[M^1M^2(L)_2(im\text{-}H)]^{3-}$	12.71	14.57	11.81
	(6.34)*	(5.77)*	(5.92)*

*pK values of hetero-binuclear complexes are shown in parenthesis

Table 6–Spectrophotometric data for copper(II) complex with salicylalanine and imidazole.

S.No.	Compositon	Complex	pH	λ_{max} (nm)	ε (dm^3mol^{-1} cm^{-1})
1.	Cu^{2+} : LH (1:1)	$[Cu(L)]^-$	6.0	688	44
2.	Cu^{2+} : LH : imH (1:1: 1)	$[Cu(L)(im)]^-$	7.0	635	50
3.	Cu^{2+} : LH : imH (2:2: 1)	$[Cu_2(L)_2(im)]^{2-}$	6.5	638	117
4.	Cu^{2+} : LH : imH (2:2: 1)	$[Cu_2(L)_2(im\text{-}H)]^{3-}$	7.5	625	130
5.	Cu^{2+} : Zn^{2+} : LH : imH (1:1:2:1)	$[CuZn(L)_2(im)]^{2-}$	6.5	635	50
6.	Cu^{2+} : Zn^{2+} : LH : imH (1:1:2:1)	$[CuZn(L)_2(im\text{-}H)]^{3-}$	7.5	623	65
7.	Cu^{2+} : Ni^{2+} : LH : imH (1:1:2:1)	$[CuNi(L)_2(im)]^{2-}$	6.5	640	44
8.	Cu^{2+} : Ni^{2+} : LH : imH (1:1:2:1)	$[CuNi(L)_2(im\text{-}H)]^{3-}$	7.5	632	47

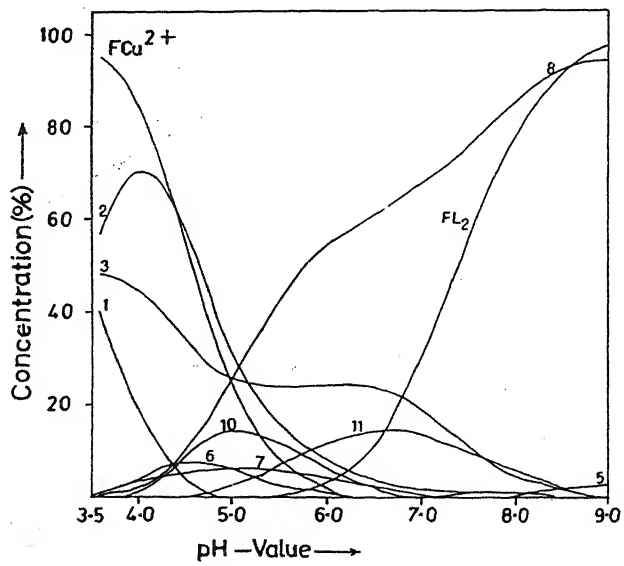


Fig. 1–Species distribution curves of 2:2:1, Cu^{2+} : LH:lmH (L=Salala) System: (1) LH_2^-, (2) LH^{2-}, (3) imH^+, (4) $Cu(OH)^+$, (5) $Cu(OH)_2$, (6) CuL^-, (7) $Cu(im)^{2+}$, (8) $Cu(L)(OH)^{2-}$, (9) $Cu(L)(im)^-$, (10) $Cu_2(L)_2(im)^{2-}$ and (11) $Cu_2(L)_2(im\text{-}H)^{3-}$

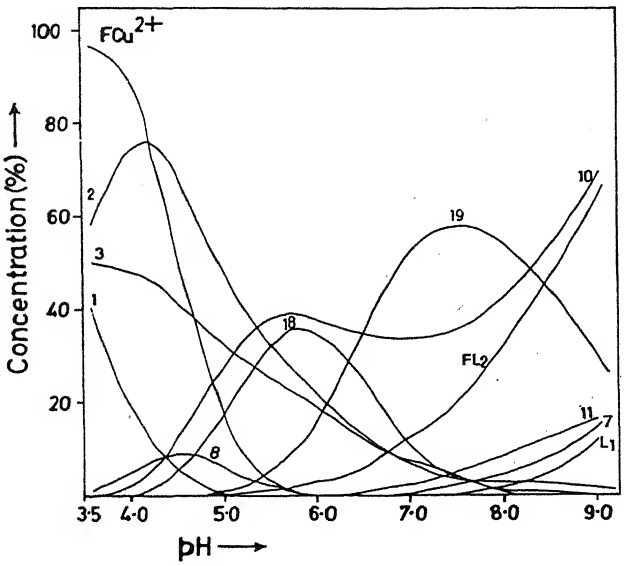


Fig. 2–Species distribution curves of 1:1:2:1, Cu^{2+} : Zn^{2+} : LH:ImH (L=Salala) System: (1) LH_2^-, (2) LH^{2-}, (3) imH^+, (4) $Cu(OH)^+$, (5) $Cu(OH)_2$, (6) $Zn(OH)^+$, (7) $Zn(OH)_2$, (8) CuL^-, (9) ZnL^-, (10) $Cu(L)(OH)^{2-}$, (11) $Zn(L)(OH)^{2-}$, (12) $Cu(L)(im)^-$, (13) $Zn(L)(im)^-$ (14) $Cu_2(L)_2(im)^{2-}$, (15) $Zn_2(L)_2(im)^{2-}$ (16) $Cu_2(L)_2(im\text{-}H)^{3-}$, (17) $Zn_2(L)_2(im\text{-}H)^{3-}$, (18) $CuZn(L)_2(im)^{2-}$ and (19) $CuZn(L)_2(im\text{-}H)^{3-}$.

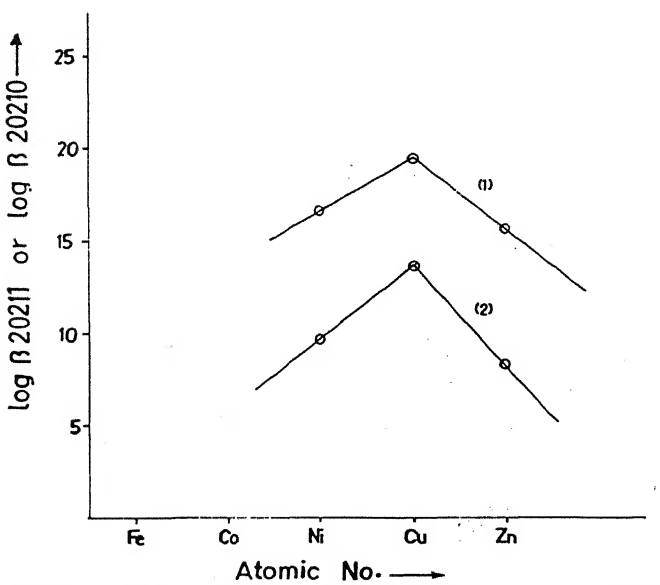


Fig. 3–Composite Irving-Williams plot of log β_{20210} and log β_{20211} : (1) $M_2(L)_2(im)^{2-}$ and (2) $M_2(L)_2(im\text{-}H)^{3-}$ [M=Cu^{II} and Zn^{II}, L= Salala].

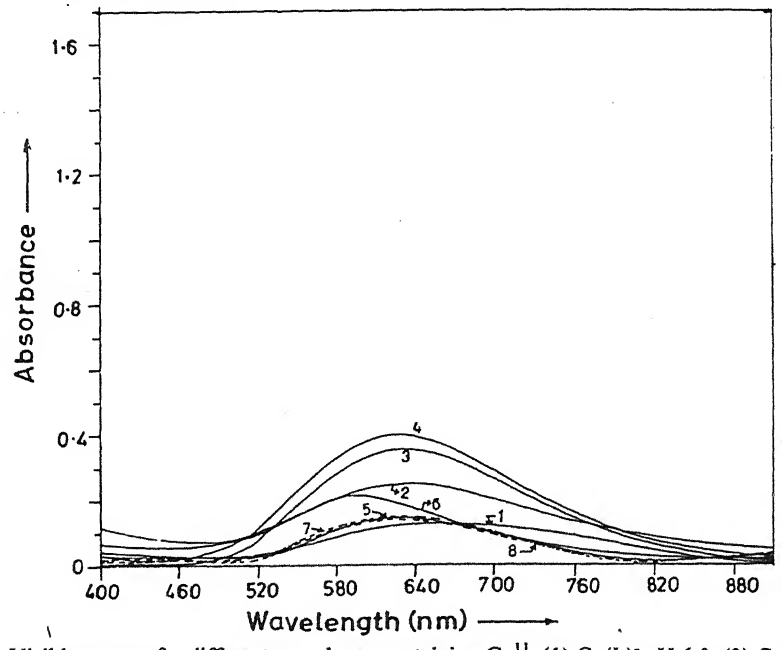
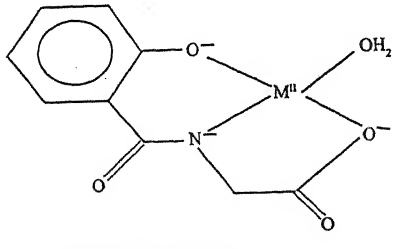
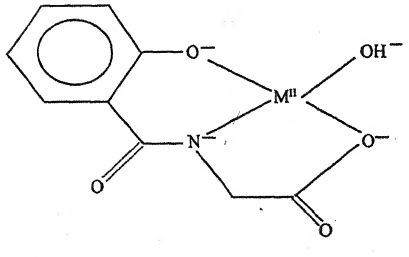


Fig. 4–Visible spectra for different complexes containing Cu^{II}, (1) $Cu(L)^{-}$ pH 6.0, (2) $CuL(im)^{-}$, pH 7.0, (3) $Cu_2(L)_2(im)^{2-}$ pH 6.5, (4) $Cu_2(L)_2(im\text{-}H)^{3-}$ pH 8.5, (5) $Cu^{-}Zn\ (L)_2(im)^{2-}$ pH 6.5, (6) $CuZn(L)_2(im\text{-}H)^{3-}$ pH 8.5, (7) $CuNi(L)_2(im\text{-}H)^{2-}$ pH 6.5 and (8) $CuNi(L)_2(im\text{-}H)^{3-}$ pH 8.5.



Structure. (I) $[ML]^{-}$



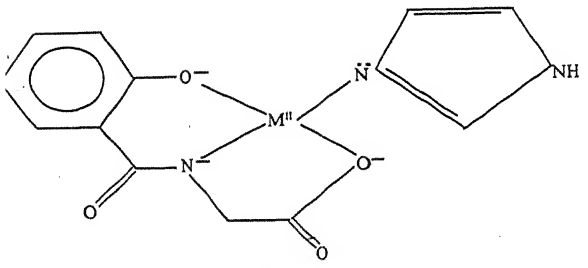
Structure (II) $[ML(OH)]^{2-}$

M(II)-Salala (1:1) system :The binary metal (II) : Salala (1:1) systems show the formation of only two $(ML)^{-}$ and $[ML)OH)^{2-}$ species. The stability constant for $M(im)^{2+}$ have also been evaluated with used three metal ions (metal ions = Cu(II) and Zn (II). The probable structure of the species ML^{-} or $[ML(OH)]^{2-}$ are given in Structure 1 and II.

The $[ML)OH)^{2-}$ species at higher pH was detected and is due to the deprotonation of aquo species $(ML)^{-}$. The overall stability constant values are reported in Tables (1-5) and some representative distribution curves are shown in Fig. (1 & 2).

M(II)-Salala-ImH (1:1:1 or 2:2:1) ternary systems : The overall stability constants and value for the ternary systems are reported in Table 4. Two kinds of ternary systems have been studied. In one kind where stoichiometry 1:1:1; remains $[ML(im)]^-$ (Str. III) mononuclear species were formed whereas in 2:2:1 stioichiometry $[M_2L_2(im)]^{2-}$ (Str. IV) type homobinuclear species were formed whereas in 2:2:1 stoichiometry $[M_2L_2(im)^{2-}$ (Structure IV) type homo-binuclear species were detected.

The pH-metric speciation curves (Fig. 1-2) indicate that complex formation starts at pH ~3.5 with a species $[M_2L_2(im)^{2-}$. The suggested most likely Structures III, IV, V are as :

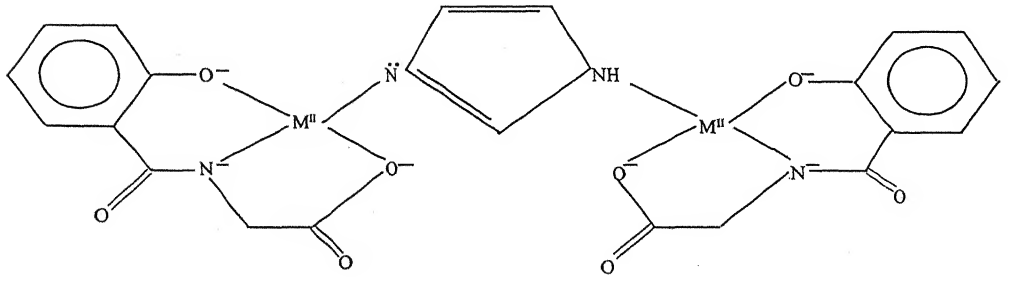


Structure (III) $[ML(im)]^-$

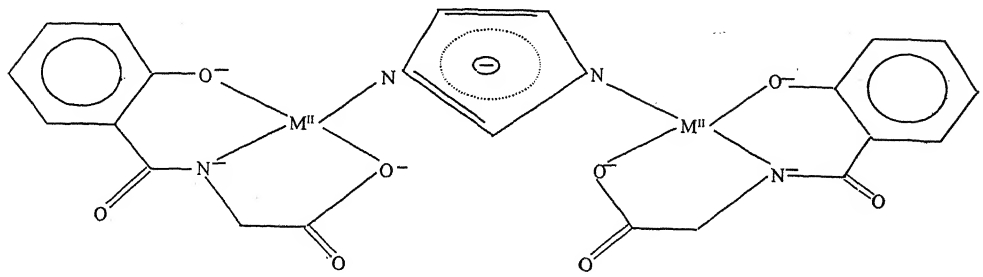
At pH > 9 the species $[M_2(L)_2(im-H)^{3-}$ is observed and is due to the deprotonation of pyrrole nitrogen of imidazole. The stability constant for this deprotonated species $[M_2)(L)_2(im-H)]^{3-}$ is lower than that of protonated species $[M_2(L_2)im]^{2-}$. The pK value of the deprotonated species was also calculated and is tabulated in Tables (4 & 5). The pK value of these species are similar to that observed in the Metal(II) : aspa : ImH (2:2:1) system[8,9].

The stability constants for hetero-binuclear $[M^1M^2(L)_2(im)]^{2-}$ of the type Cu-Zn/Cu-Ni/Ni-Zn have also been studied (Fig. 2). Again deprotonation constants of these hetero-binuclear complexes were observed. The pK values are identical with that of reported pK values for similar systems[8,9].

Stability constants of various binary and ternary species follow the Irving-Williams order[13,14]. The overall stability constants of the binuclear species form a composition curve (Fig. 3). Overall stability constants of binuclear complexes form a composite Irving-Williams order and this trend is found to be as :



Strucutre (IV) $[M_2L_2(im)]^{2-}$



Structure (V) $[M_2(L)_2(im\text{-}H)]^{3-}$

CuCu>CuNi>CuZn>NiNi>ZnZn>. The stability constant of Ni-Ni complexes are found to be less than Ni-Zn complex.

Optical absorption spectra : The copper binding sites in each of the complexes identified in the potentiometric studies were determined spectrophotometrically. The electronic spectra also afforded elucidation of the solution structures of the complexes. The solutions similar to those in the potentiometric studies were carried out using similar concentration ranges metal to ligand ratios and covering the similar pH ranges. The absorption spectra obtained for the individual species are shown in Fig. 4. The λ_{max} values (Table 6) agree resonably well with the respective values published for copper(II):aspa:ImH system[8,9]. In each case a single absorption band was observed which can be assigned to the spin-allowed laporte forbidden *d-d* transition, $^2E_g \rightarrow {}^2T_{2g}$. Since this is a d^9 system, the crystal field splitting is in turn influenced by the coordination sphere of the metal ion and hence λ_{max} affords a measure of the solution structure of the complex. For the composition Cu^{2-}:HL (1:1) the dominating species $[CuL]^-$ at pH 6.0 shows λ_{max} at 688 nm having extinction coefficient 44 $dm^3mol^{-1}cm^{-1}$. The corresponding ternary species with imidazole at pH 7.0 yields the λ_{max} at 635 nm. The ~53 nm shift in λ_{max} is due to fourth ligand imidazole. This is due to the imidazole which exerts higher ligand field than water molecule.

The λ_{max} value (with ε_{max}=117) of $[Cu_2(L)_2im]^{2-}$ species is virtually the same as that of the $[M(L)(im)]^-$ species, indicating the same coordination as $[ML(im)]^-$ species. Furthermore, only one absorption maximum is observed, indicating a similar environment for the two copper (II) ions. On raising the pH of the same system ~13 nm shift (blue shift) is observed. This is due to the reason that the resulting anion (im-H)

obviously exerts a strong ligand field than the neutral imidazole. The λ_{max} values of $[CuZn(L)_2im]^{2-}$/$[CuNi(L)_2im]^{2-}$ species was again identical to that of $[CuL(im)]^{-}$indicating the presence of same geometry around Cu(II).

These hetero-binuclear species also show blue shift due to presence again (im-H) bridging moiety. Further evidence supporting these solution spectra is given by earliear workers[15-17]. They have also collected the similar spectrophotometric data for the copper(II) binuclear systems.

Authors thank U.G.C. New Delhi for financial assistance.

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Preparation of diaryldiazabutadienes from benzaldehyde hydrazones using bis (acetylacetonato) copper(II)

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Abstract An extremely fast reaction of the benzaldehyde hydrazones with a catalytic amount of bis(acetylacetonato)-copper(II) affords diaryl-diazabutadienes in quantitative yields. The compounds have been characterized by satisfactory analytical and spectral data and a tentative mechanism of their formation through oxidation of hydrazones is described.

(**Keywords** : hydrazones/$Cu(acac)_2$/oxidation/ carbenoids/diazabutadienes)

Diazabutadienes have drawn considerable interest of the chemists as many of their uses have been subjects of patent[1]. Their applications range from electrochemistry to medicinal chemistry and as synthetic reagents for some novel heterocyclic compounds[2-4]. The conventional methods for the preparation of diazabutadienes involve either the reaction of hydrazones with aldehydes or the treatment of hydrazones with acidds like H_2SO_4, PPA in times ranging from 45 min. to several hours.[5]. Recently, synthesis of some asymmetrical azines has been reported from semicarbazones and aldehydes[6]. Most of these methods are under vigorous reaction conditions and time consuming and need to be improved. The present communication reports an extremely fast reaction of benzaldehyde hydrazones (1a-d) with a catalytic amount of bis(acetylacetonato)copper (II) leading to the formation of 1,4-diaryl-2, 3-diaza-1,3-butadienes (2a-d) in almost quantitative yields. It is noteworthy to mention here that $Cu(acac)_2$ has been used widely by carbene chemists to catalyze the decomposition of diazo compounds and generate carbenoids[7].

The products have been characterized on the basis of comparison (CO – IR and undepressed mixed mp) with an authentic sample[5] and satisfactory analytical (Table 1) and spectral (^1H and ^{13}C NMR and Mass; Table 2) data. Both ^1H and ^{13}C NMR spectra confirm the symmetrical nature of molecules by showing the equivalence of two azomethine carbons and protons and also of aromatic carbons and protons of one aromatic ring to other. The ^{13}C NMR spectrum of 2c shows two comparatively downfield signals (δ 162.38 and 161.51) instead of one shown by ^{13}C NMR spectra of 2a-c. The

^{13}C –proton coupling study shows that signal at δ 162.38 is a singlet and has been assigned to methoxy substituted aromatic carbons. The signal at δ 161.51 is a doublet and has been assigned to azomethine carbons.

Table 1.– Physical data of diaryldiazabutadienes (2a-d)

S.No.	m. p. (^{0}C)	Mol. formula	C	H	N
			(Required %)		
2a	92	$C_{14}H_{12}N_2$	80.49	6.02	13.18
			(80.77	5.77	13.46)
2b	168	$C_{16}H_{16}N_2$	81.06	7.05	11.57
			(81.36	6.78	11.86)
2c	181	$C_{16}H_{16}N_2O_2$	71.25	6.22	10.12
			(71.64	5.97	10.44)
2d	220	$C_{14}H_{10}N_2Cl_2$	60.44	3.88	09.88
			(60.87	3.62	10.14)

Table 2.– NMR and mass spectral data of diaryldiazabutadienes (2a-d)*

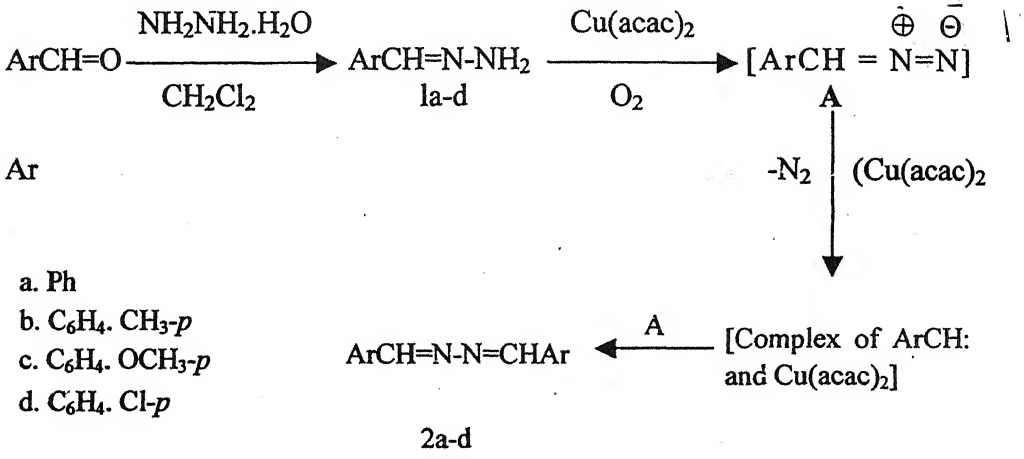
S. No.	^{1}H NMR ($CDCl_3$· δ ppm)	^{13}C NMR** ($CDCl_3$ δ ppm)	MS *m/z* (r.i.)
2a	8.70 (s, 2H, azomethine), 7.91-7.81 (m, 4H, arom.), 7.55-7.41 (m, 6H, arom.)	162.55 (azomethine C), 134.47 (C–1), 131.64 (C–4), 129.21 (C–2), 128.98 (C–3).	208 (M^+, 100), 180 (40, M^+–N_2), 131 (25, M^+ –Ph,), 104 (2, PhCH= N^+), 77 (5, Ph)
2b	8.66 (s, 2H, azomethine), 7.75-7.28 (four dd, 8H, arom.), 2.43 (s, 6H, methyl)	162.30 (azomethine C), 142.02 (C–4), 131.86 (C–1), 128.92 (C–2), 22.05.	236 (M^+, 85), 208 (35, M^+–N_2), 145 (100, M^+ –*P*–tolyl), 118 (26, Me-C_6H_4–CH= N^+), 91 (40, $CH_3C_6H_4$)
2c	8.60 (s, 2H, azomethine), 7.80, 7.78, 6.99, 6.95 (four dd, 8H, arom.), 3.86 (s, 6H, methoxy)	162.38 (C-4), 161.51 (azomethine C), 130.52 (C–2), 130.52 (C–2), 127.37 (C–1), 114.64 (C-3), 55.79).	238 (M^+, 96), 240 (20, M^+–N_2), 161 (100, M^+ –*P*–anisyl), 134 (48, MeO-C_6H_4–CH=N^+), 107 (14, MeO.C_6H_4)
2d	8.60 (s, 2H, azomethine), 7.80, 7.75, 7.45, 7.37 (four dd, 8H, arom.),	161.00 (azomethine C), 137.75 (C–4), 132.86 (C–1), 130.15 (C–2), 129.56 (C-3).	276 (M^+, 100), 248 (10, M^+–N_2), 165 (95, M^+–C_6H_4. Cl), 138 (40, ClC_6H_4-CH=N^+), 111 (48, C_6H_4.Cl).

*The NMR spectra have been recorded on a Bruker™ 300 MHz spectrometer in a $CDCl_3$ solution and mass spectra are recorded on a Mat SSQ 7000 spectrometer using EI method.

**C-1 is aromatic carbon attached to azomethine carbon while C-2, C-3 & C-4 are *o*-, *m*- & *p*- to it, respectively.

In a typical reaction procedure, hydrazine hydrate (5 mmol) is added to a dichloromethane solution (10 ml) of an appropriate aldehyde (5 mmol) in a 100 ml round bottomed flask and stirred at room temperature for 5 min. The solution is kept over anhydrous sodium sulfate for 15 min. and then decanted to another round bottomed flask. $Cu(acac)_2$ (0.5 mmol) in 10 ml of dichloromethane was added slowly during 5 min. time to the hydrazone solution. After stirring for 5 min. at room tempera-ture the reaction mixture was filtered through neutral alumina. Evaporation of the solvent under reduced pressure afforded yellow solids in quantitative yields which were recrystallized from ethanol.

The most plausible mechanism for the formation of products involves $Cu(acac)_2$ – catalyzed oxidation of benzaldehyde hydrazones formed initially to the corresponding diazoalkanes. The latter may react in the usual way like the carbenoids do (Scheme 1) with another molecule of diazoalkane to afford the final product[7]. The aryldiazoalkanes could not be isolated even at 0^0C due to their extremely reactive nature. However, their intervention is ascertained by isolation of α-diazoketones by oxidation of benzil monohydrazones using $Cu(acac)_2$[8].



Scheme 1

In conclusion, this catalytic reaction is a clean, convenient and quickest method for the synthesis of diazabutadienes.

We are grateful to Professor B.M. Abegaz, Head of the Chemistry Department, University of Botswana for providing the necessary research facilities.

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Solution equilibria of mixed ligand complexes of vanadium (IV) with glycylglycine and imidazoles

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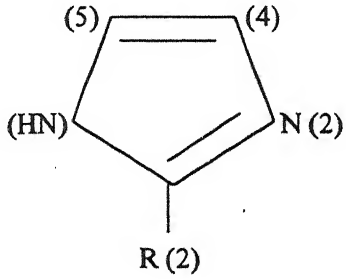
Abstract Solution equilibria of mixed ligand complexes of VO^{2+} with glycylglycine and imidazoles are investigated in aqueous solution by means of potentiometry, spectrophotometry and electron paramagnetic resonance spectroscopy. The stability constants of binary and ternary complexes have been determined at 25 ±1°C in 0.1M $NaClO_4$. The experimental data have been obtained for the following species : (GG^-= glycylglycinate and Im= imidazole) VO(GGH), VO(Im), VO(GGH_{-1}), VO(GG)(ImH) and VO(GG)(ImH_{-1}) along several hydrolysis products. The involved functional groups of various species are also predicted and discussed.

(**Keywords :** mixed ligand complexes/solution equilibria/epr/glycylglycine/imidazoles)

Vanadium is an essential nutrient for higher animals[1], although this has not yet been clearly established as such to human life[2]. It can act, for instance, as an enzyme regulator in various phosphate metabolizing reactions, suggesting binding between vanadium and protein side chains[3]. Its interaction with peptides in physiological pH range, *via*; coordination of the deprotonated oligopeptide-N^- group, have been proved to involve vanadium in oxidation state[4–6]. VO^{2+} forms complexes of fairly high stability with ligands containing O-donor atoms, but it binds more weakly to N^-or S^- donor containing biogenic ligands[7] in solution. Imidazole is bound to metal ions as a histidine moiety in several metalloproteins, where in peptides are the other ligands. In view of this fact, peptide-VO^{2+}-imidazole ternary systems are important biological entities. Interaction among VO^{2+} and glycylglycine (Glygly) were first investigated by Pessoa *et al*[8]. A review of literature showed that very little equilibrium study on VO^{2+} : Glygly : Im has been reported[9,10]. With this view in mind we have studied the formation of ternary complexes for the system VO^{2+} Glygly : ImH (1:1:1) with three different imidazoles (I) *viz.* Imidazole (ImH), 2-methylimidazole (2-M-ImH) and 2-ethylimidazole (2-E-ImH) with a view to see the effect of alkyl substitutions on the stability of the ternary complexes.



(a) R =H
(b) R = –CH_3
(c) C = –CH_2CH_3

All chemicals used were of A.R. grade. The standard solutions were prepared by using double-distilled water. Metal ion solutions were standardized by the usual procedure[11].

The CO_2 free NaOH solutions were prepared under an atmosphere of nitrogen. It was kept in polyethylene bottle with sodalime guard tube. All solutions were made up to an ionic strength of 0.1M $NaClO_4$.

Protonation constants of the ligands and stability constants of the vanadium (IV) complexes were determined in a 0.1 M $NaClO_4$ at 25 °C by using a Systronics 335 pH-meterusing a special glass electrode (accuracy ±0.01 pH) in conjun-ction with a saturated calomel electrode. The general procedure for pH-metric titration was the same as described elsewhere[12-17]. The total volume of the solution was kept 50 ml. The solution in the vessel was stirred continuously by using magnetic stirrer. The temperature was maintained using a Yorko thermostat. For the ternary systems following sets of solutions were prepared (in total volume 50 ml) for titrations :

(1) 0.03M perchloric acid + 0.1M $NaClO_4$
(2) 0.03M perchloric acid + 0.003M Glygly + 0.1M $NaClO_4$
(3) 0.03M perchloric acid + 0.003M Im + 0.1M $NaClO_4$
(4) 0.03M perchloric acid + 0.003M VO^{2+} + 0.003M Glygly + 0.1M $NaClO_4$
(5) 0.03M perchloric acid + 0.003M VO^{2+} + 0.003M Im + 0.1M $NaClO_4$
(6) 0.03M perchloric acid + 0.003M VO^{2+} + 0.003M Glygly + 0.003 M Im + 0.1M $NaClO_4$.

Each one of the above sample was titrated against 1.0M sodium hydroxide.

The epr spectra were recorded with a Varian E-line Century Series Spectrometer, equipped with a dual cavity and operating at X-band 100 kHz modulation. TCNE was used as field marker. The $g_{\|}$ and $A_{\|}$ values were measured according to the standard procedure[18,19]. The visible absorption spectra were recorded on a Systronics UV-vis recording Spectrophotometer UV-117 with 1 cm quartz cell in aqueous medium. The vanadium binding sites in each of the ternary complexes were determined spectrophotometrically. Electronic configuration of vanadyl ion (VO^{2+}) allows visible and epr spectra,

which provide information about the ligand field strength and indirectly of the donors to the vanadium, to be readily measured. The electronic spectra also afforded elucidation of the solution structures of the complexes. Similar sets of solution to those in the potentiometric studies were carried out using similar concentration ranges of ligands and VO^{2+} and covering similar pH range.

The protonation constants of glycylglycine and imidazoles were evaluated by Calvin-Bjerrum's[20-21] technique as adopted by Irving and Rossotti[22] and imidazoles are presented in Table 1. The stability constants for the binary and ternary systems were evaluated using SCOGS computer programme[23] and the values are given in Table 2.

Table 1– Protonation constants ($\log\beta_{oorst}$) of glycylglycine and imidazoles at 25±1°C and I = 0.1M $NaClO_4$.

Complex	$\log\beta_{oorst}$
GGH_2^+	11.23
GGH	8.05
ImH^+	7.1 (8.0)[a]

a = value of logβ for 2-M-ImH/2-E-ImH

Table 2–VO^{2+} ($\log\beta_{oqrst}$) stability constants for the binary and ternary complexes at 25±1°C and I=0.1 M $NaClO_4$.

Complex	$\log\beta_{oqrst}$
VO(GGH)	7.30
$VO(GGH_{-1})$	2.52
VO(Im)	5.77
VO(GG)(Im)	12.88 (14.41)[a]
$VO(GG)(ImH_{-1})$	1.00

a = value of logβ for 2-M-ImH/2-E-ImH

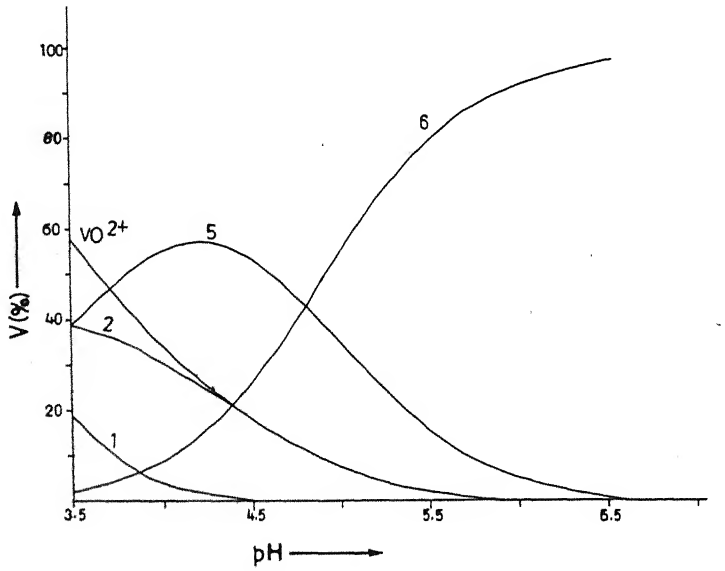


Fig. 1 – Species distribution curves of VO^{2+} : Glygly (1:1) system : (1) GGH^{+}_{2}, (2) GGH, (3) $VO(OH)^{+}$, (4) $VO(OH)_2$, (5) VO(GGH) and (6) $VO(GGH_{-1})$.

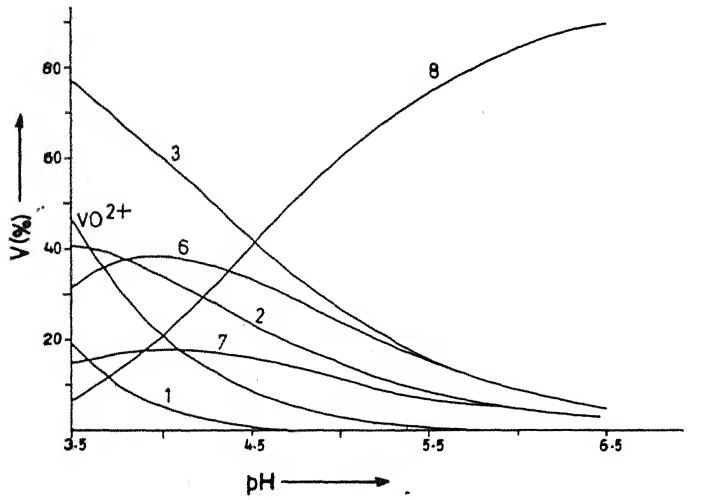


Fig. 2 – Species distribution curves of VO^{2+} : Glygly : ImH (1:1) system : (1) GGH^{+}_{2}, (2) GGH, (3) ImH^{+}, (4) $VO(OH)^{+}$, (5) $VO(OH)_2$, (6) $VO(GGH_{-1})$, (7) VO(Im), (8) VO(GG)(Im) and (9) $VO(GG)(ImH_{-1})$.

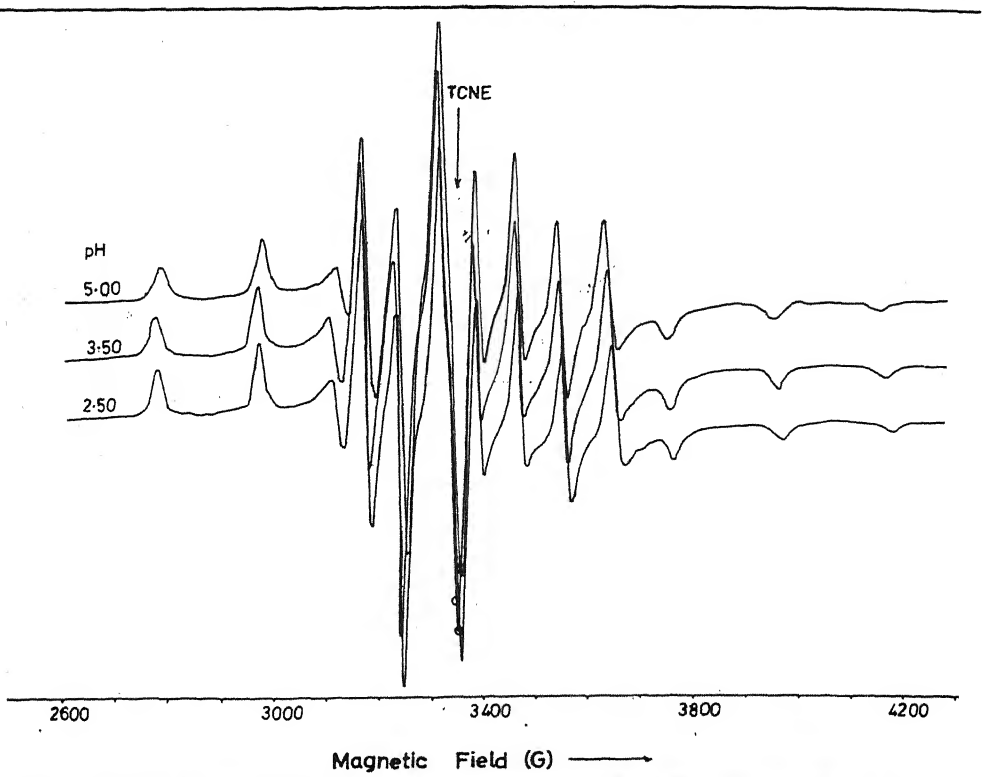


Fig. 3 – X-band epr spectra of VO^{2+} : Glygly : ImH (1:1:1) ternary system as a function of pH.

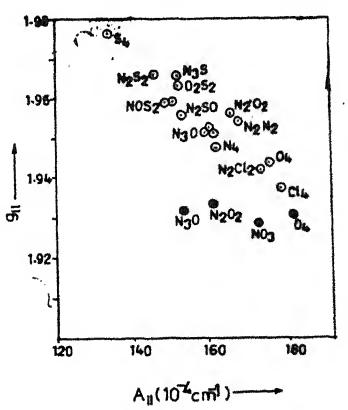


Fig. 4 – A correlation plot of $A_{\parallel}$ *vs.* $g_{\parallel}$ for the VO^{2+} complexes : (•) NO_3, N_2O_2, N_3O, O_4 and (o) a series of oxovanadium (IV) complexes with various equatorial donor atoms [24].

The glycylglycine shows two replaceable protons : pK_{a1}=3.00 and pK_{a2}=8.10 and imidazoles show only one proton (pK_a = 7.1 for ImH, and pK_a = 8.00 for 2-M-ImH/2-E-ImH). These values compare with literature values[23]. The species distribution curves (representative curves) are shown in Fig.(1 & 2). The binary species VO(GGH) have abundance ~ 40% at pH 3.50. The distribution curves (Fig. 1) indicate that the deprotonated binary species $VO(GGH_{-1})$ starts at pH ~ 3.50. In this species the peptide bond could be deprotonated. On seeing distribution curves of the VO^{2+} : Glygly : ImH ternary system it is clear that the ternary species have the abundance ~ 7% (Fig. 2) at pH 3.50 which is indicative of ternary complex formation.

In relating the molecular structure of an aqueous species to its stability, it is generally useful to compare a spectroscopic property with that of crystalline solid of known structure. Unfortunately, in our present studies of peptide complexes with this metal ion, there are no reportings on crystal structures. So our discussion of the structures of these species is based on their properties in solution. The donor atoms with this peptide are amino-N, amide-N and carboxylate-O. The order of logβ values with respect to imidazoles is : 2-methylimidazole ≥ 2- ethylimidazole > imidazole. As we go from imidazole to 2-methylimidazole, an increase in logβ values (Table 2) is observed, which is owing to the increase in basicity of ligands[12,13]. From steric considerations the logβ values in the case of complexes with substituted imidazoles should be smaller than that for imidazole complexes. The increase in basicity in substituted imidazoles, thus, compensates for the negative contribution of the steric effect and further enhances the value of logβ in all the cases. Amongst the substituted imidazoles, there is not much difference either in the ligand basicities or in the steric effect, so that both, 2-methylimidazole and 2-ethylimidazole, give almost similar logβ values.

We have also recorded UV-visible and epr spectra of the above systems in the same conditions as were in potiometric study. The result of spectroscopic studies tallies well to that of potentiometric data. Some representative epr spectra are shown in Fig. 3 and derived epr parameters are given in Table 3. In the same table, involved functional groups along the estimated epr parameters are also given. The binary systems at pH 2.50 may have the coordinated functional group, only $4H_2O$ and above this pH the involved functinal groups are : $R\text{-}NH_2$, $R\text{-}CO_2$, H_2O and CONH. Up to pH 2.5 the $g_{\|}$ and $g_{|}$ values agree well to those of experimental one (Table 3). Above pH 2.50, containing $VO(H_2O)^{2+}{}_5$ the binary VO^{2+} : Glygly system gives rise to the species VO(GGH) having coordination sites : $R\text{-}NH_2$,$R\text{-}CO_2$, H_2O and CONH. Similary ternary systems at pH 2.5 comprises of the aquo VO^{2+} and above

Table 3 – EPR parameters, proposed equatorial coordination sites and λ_{max} for vanadyl(IV) binary and ternary systems.

S. No.	Composition	Complex	pH	$g_{\|}$	$g_{\perp}$	$A_{\|}$ (10^{-4} cm^{-1})	$A_{\perp}$ (10^{-4} cm^{-1})	Proposed equatorial coordination	$A_{z,i}$, 10^{-4} cm^{-1} Calculated	Literature	λ_{max} (nm) (in H_2O)
1.	VO^{2+} : Glygly (1:1)	VO^{2+}aq.	2.50	1.931	1.974	183	73	$4H_2O$	45.9	45.7	628,770
		VO(GGH)	3.50	1.929	1.979	174	58	R-NH_2	39.9	40.1	610,765
								R-CO_2^-	42.5	42.7	
								$2H_2O$	45.5	45.7	
		$VO(GGH_{-1})$	5.00	1.934	1.978	160	50	R-NH_2	39.6	40.1	
								R-CO_2^-	42.2	42.7	
								H_2O	45.2	45.7	
								CONH	31.5	32.0	
2.	VO^{2+} : Glygly : ImH (1:1:1)	VO^{2+}aq	2.50	1.911	1.981	183	71	$4H_2O$	45.9	40.1	615,770
		VO(GG)(Im)	3.50	1.931	1.981	153	41	R-NH_2	40.0	40.1	610,765
								R-CO_2^-	42.6	42.7	
								=N-	38.2	38.3	
								CONH	31.9	32.0	
		VO(GG)(Im)	5.00	1.932	1.978	153	41	R-NH_2	40.0	40.1	
								R-CO_2^-	42.6	42.7	
								=N-	38.2	38.3	
								CONH	31.9	32.0	
3.	VO^{2+} : Glygly : M-ImH/E-ImH : (1:1:1)	VO^{2+}_{aq}	2.50	1.931	1.987	183	69	$4H_2O$	45.9	47.7	640,760
		VO^{2+}_{aq} VO(GG)(Im)	3.50	1.931	1.980	183	70	$4H_2O$	45.9	45.7	610,765
		VO(GG) (Im)	5.00	1.933	1.980	153	41	R-NH_2	40.0	40.1	
								R-CO_2^-	42.6	42.7	
								=N-	38.2	38.3	
								CONH	31.9	32.0	
		VO(GG) (Im)	6.00	1.931	1.980	153	40	R-NH_2	40.0	40.1	575
								R-CO_2^-	42.6	42.7	
								=N-	38.2	38.3	
								CONH	31.9	32.0	

this pH (i.e. pH 3.50) the epr parameters are different than those of the pH values of binary one. Hence, it is assumed that the decreased values of $A_{\|}$ (i.e.; 153G) is definetly due to different species comprises following sites : R-NH_2, R-CO_2^- = N- and CONH. The estimated spin Hamiltonian parameter are given in Table 3. On seeing systems having 2-M-ImH/2-E-ImH it is clear that the ternary complex formation takes place at pH~5.0.

We have made a correlation plot (Fig. 4) between $g_{\|}$ and $A_{\|}$ for a series of known oxovanadium(IV) complexes along with the present complexes containing various equatorial donor atom sets[24]. The data lie approximately on a straight line with negative slope. This anticorrelation between $g_{\|}$ and $A_{\|}$ is well known and simply indicates that increased in plane π- and σ- bonding results in decreased $A_{\|}$ values and increased $g_{\|}$ values[24]. On addition of our data on the same plot reveals that our data points deviate substantially from the locus of the reference data. The shift of our data points as well as relative to reference data, implies that the physical mechanism which determines the $A_{\|}$ values in our data is not the same as in the reference complexes.

The visible spectra for VO^{2+} : Glygly : ImH (1:1:0/1) as pH is raised resemble those for epr spectral data. Upto pH 3.50 both binary and ternary systems show two distinct bands (Table 3). Above pH 3.50 the visible bands are ill defined. At high pH values the λ_{max} of the observed bands shifted to higher energy band positions. This increased ligand-field suggests corresponding binary/ternary complexes are formed as the pH is raised.

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Novel coated wire copper (II) and zinc (II) ion-selective electrodes: their application in the analysis of brass

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Abstract Two new coated wire Cu(II) ISE and Zn(II) ISE were fabricated using Cu(II) cupron complex and Zn(II) rhozonate as electroactive material. The characteristics of both the electrodes have been studied to evaluate their utility. The electrodes were used as indicator electrode in potentiometric titration for determining the concentration of respective ions, the application of these electrodes were further demonstrated by determining the copper and zinc contents of brass samples.

(**Keywords :** coated wire Cu(II) ISE / coated wire Zn(II) ISE / analysis of brass)

Ion-selective electrodes (ISE's) have become one of the most useful tools for rapid analysis. Many modern techniques have come into existence for the analysis of alloys[1,2]. The biggest disadvantage of the latest instrumental techniques are their high operating cost and their availability in only sophisticated laboratories. The main appeal of ISE's lie in their simplicity of measuring techniques and instrumentation, easy and cheap availability of these equipments.

Although a number of ISE's for Cu(II)[3] and Zn(II)[4] ions are known, they follow the traditional barrel configuration and require not only large sample size but also have to be kept in upright position for the measurement. Freiser and coworkers[5] have developed coated wire electrodes for several ions, which only require 1-2 mm in diameter of electrode and can be used at any angle with low cost. However, they have not developed any coated wire electrode for either Cu(II) ion or Zn(II) ion.

In this work, new coated wire ion selective electrodes for Cu(II) and Zn(II) ions have been fabricated, their characteristics were studied and these electrodes were used for the estimation of constituents of brass.

Fabrication of the electrode : A dirty green coloured precipitate of Cu(II)-cupron complex was obtained by mixing solutions of $CuCl_2$ and cupron (α-benzoinoxime)[7]. Similarly, a reddish brown precipitate of Zn(II)-rhodizonate was obtained by mixing solutions of $ZnCl_2$ and neutral rhodizonic acid.[8]

The filtered precipitates were washed thoroughly and dried in air at room temperature. The precipitates obtained were used as electroactive materials for fabrication of respective electrodes.

The separate coaxial copper cable was coated by dipping it several times in the slurry

of respective electroactive material and PVC in tetrahydrofuran until a bead was formed at the tip. The cables along with the bead at their tips were used as respective Cu(II) ISE and Zn(II) ISE which were dried in air for 48 h.

Titration of Cu(II), Zn(I) ions against phosphate ions : Both Cu(II) and Zn(II) give precipitate with orthophosphate ion and precipitation titration between these species can be monitored with suitable sensors. For the titration of the above metal ions against phosphate ions, standard solutions of the salts of Cu(II) and Zn(II) (0.01 mol dm^{-3}) and Na_2HPO_4 (0.01 mol dm^{-3}) were prepared separately. The solution (5 ml) was diluted to 20 ml by double distilled water and e.m.f. measurements were made while titrating this solution against Na_2HPO_4 using respective ISE. The e.m.f. values were plotted against the volume of Na_2HPO_4 solution consumed in titration. A sharp rise in the titraton curve was observed to occur near the end point in both the cases.

Determination of Cu and Zn in the brass sample : Sample solutions were prepared as follows:

0.1 g, 0.2 g, 0.3 g, 0.4 g, and 0.5 g were weighed and following treatments were given to each of them. The samples were dissolved in aqua regia and the resultant liquid was heated over sand bath. The residue was extracted with distilled water. The impurities were separated by filtration. The filtrate was treated with sulfuric acid and evaporated to fumes to expel nitric acid. The residual liquid was evaporated to dryness, cooled and treated with distilled water. After filtration it was made up to a volume of 100 ml. E.m.f. measurements were made for each solution using Cu(II) ISE and the amount of copper was calculated from the calibration curve. (Table 1).

Sample solutions were prepared again weighing 0.3 g, 0.5 g, 0.7 g, 0.9 g and 1.0 g and treated as above, further, it was treated with ammonium thiocyante (less than 0.05N) to precipitate copper from the filtrate. The filtrate and washings collected after filtration were heated to nearly boiling and further treated with concentrated ammonium hydroxide until a permanent precipitate was obtained. This was again filtered and the filtrate and washings collected were boiled and evaporated to a minimal amount. The residual liquid was cooled and taken up with distilled water and made up to 100 ml. E.m.f. measurements were made for each solutions using Zn(II) ISE. The amount of zinc was calculated from calibration curve (Table 2).

Two separate series of standard solutions (1×10^{-1} to 1×10^{-7} mol dm^{-3}) of $CuCl_2$ and $ZnCl_2$ were prepared and e.m.f. measurements were made using a Philips PR 9405 potentiometer at room temperatue (25±1°C). Each fabricated electrode was used as indicator electrode and saturated calomel electrode (SCE) was used as external reference electrode. The entire electrochemcial cell can be represented as:

Metal Conductor wire	Ion-selective Electrode bead	Sample solution	External Reference Electrode (SCE)

Prior to the measurements, the electrodes were conditioned overnight in 0.01 mol dm^{-3} $CuCl_2$ and $ZnCl_2$ respectively for 24 h.

Table 1 – Estimation of Cu in brass sample.

Sl. No.	Weight of Brass (g)	Standard value (amt. of Cu present in brass) (g)	Observed value (amt. of Cu present in brass) (g)
A	0.1	0.0608	0.0596
B	0.2	0.1216	0.1206
C	0.3	0.1824	0.1778
D	0.4	0.2432	0.2413
E	0.5	0.3040	0.2921

Table 2 – Estimation of Zn in brass sample.

Sl. No.	Weight of Brass (g)	Standard value (amt. of Zn present in brass) (g)	Observed value (amt. of Zn present in brass) (g)
A	0.3	0.096	0.0914
B	0.5	0.1600	0.1567
C	0.7	0.2400	0.2089
D	0.9	0.2880	0.2742
E	1.0	0.3200	0.3132

The Cu(II) ISE showed linear response down to a Cu(II) ion concentration of 1.0×10^{-5} mol dm^{-3}, with a slope of 40 mV per decade change in Cu(II) ion concentration, a response time of 10 s and working pH range of 2-5. Selectivity coefficient values determined using mixed solution method[6] showed that the sensor system is selective towards Cu(II) ions in the presence of the cations: Pb(II), Ca(II), Fe(III), Al(III), Ni(II) and Mn(II).

The Zn(II) ISE gave good response to Zn(II) ions with a slope of 40 mV per decade change in Zn(II) ion concentration and a lower detection limit of 5.0×10^{-5} mol dm^{-3} with the working pH range of 3-8 and response time of 10 s. Selectivity coefficients determined using mixed solution method[6], reveal that the electrode is selective in presence of following cations : Cu(II), Pb(II), Fe(III) and Al(III).

The utility of the electrodes was tested by using them as indicator electrodes in potentiometric titrations. The results obtained were very satisfactory. Further attempts were made to explore the possibility of applications of these fabricated electrodes in the analysis of brass. The sample used in the present work is high tensile brass (No. 10g) from Bureau of

Analysed Sample Ltd., Cleveland. The values obtained by ISE method and the standard values showed a fairly good agreement thus establishing the successful application of these fabricated electrodes.

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On the analysis of thermoluminescence glow curve

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Abstract A method has been suggested for the analysis of thermoluminescence (TL) glow curve. Area enclosed in the TL glow curve is found to be proportional to concentration of trapped carriers. Suggested method helps in the evaluation of TL decay parameters and the order of kinetics involved in the process.

(**Keywords :** thermoluminescence/thermally stimulated process/TL glow curve)

An electron excited to conduction band from valence band returns to valence band after staying for its life time in conduction band. However, if the electron in its return to valence band is trapped in the metastable state (or trap level), it needs an energy to be raised to the conduction band. In the metastable state the electron can stay for a longer time till it has a chance to receive an energy. When the system is heated, the electrons are thermally released from their respective trap centers. Such released electrons may quickly recombine with an oppositely charge centers resulting in the appearence of a thermoluminescence (TL) glow curve. The intensity of a TL glow curve with monomolecular kinetics (i.e. first order kinetics) is expressed by[1]

$$i_1 = n_0 s_1 \exp\left[(-E_{a1}/kT) - (s_1/b)\int_{T_0}^{T} \exp(-E_{a1}/kT')\,dT'\right] \quad (1)$$

where n_0 represents the initial concentration of trapped carriers per unit volume, s_1 the escape frequency factor or pre- exponential factor, E_{al} the trap depth or activation energy, k the Boltzmann's constant, b the linear heating rate, T_0 the absolute temperature at which TL glow curve starts to appear, T an arbitrary temperature in the range T_0 to T and suffix 1 represents the data related to first order kinetics. It has been pointed out by Randall and Wilkins[2] that the first order kinetics is a recombination dominant process. In the situation when probabilities of recombination and retrapping are equal[3], one observes a TL spectrum with second order kinetics. Corresponding intensity of TL glow curve is represented by

$$i_2 = n_0^2 s_2 \exp[(-E_{a2}/kT)] \times [1 + (n_0 s_2/b)\int_{T_0}^{T} \exp(-E_{a2}/kT')\,dT']^{-2} \quad (2)$$

where pre-exponential factor s_2 has the dimension m^3s^{-1}. Suffix 2 represents the parameters asociated with second order kinetics. s_2 and s_1 are found to be related as

$$s_1 = N s_2 \quad (3)$$

where N is the concentration of total electron traps per unit volume. In the situations when all the available electron traps are filled initially i.e. when $N = n_0$, the intensities of TL glow curves involving second and higher order kinetics can be represented by[4]

$$i_l = n_0 s_l \exp[-(E_{al}/kT)] \times [1 + \{s_l (l-1)/b\} \int_{T_0}^{T} \exp(-E_{al}/kT')\, dT']^{[-l/(l-1)]} \quad (4)$$

where l is order of kinetics involved. It is obvious that for l=2, eqn. (4) changes to eqn. (2) to represent the TL intensity of second order glow curve. Eqn. (4) fails to represent the TL intensity of first order glow curve. To overcome this shortcoming, an attempt has been made by Prakash[5] to modify Adirovitch set of equations. Consequently, a more generalised equation has been developed as[6]

$$i_l = \{1/(2l-1)\}\, n_0 s_l \exp[(-E_{al}/kT) - \{l s_1/(2l-1)b\} \int_{T_0}^{T} \exp(-E_{al}/kT')\, dT'] \quad (5)$$

Thus, TL intensities of higher order kinetics including first order can be represented with the help of eqn. (5).

In the derivation of eqn. (5) it has been shown by Prakash and Prasad[6] that i_l may be expressed as

$$i_l = -(1/l)\,(dn/dt)$$

and

$$(dn/dt) = -\{l/(2l-1)\}\, n s_l \exp(-E_{al}/kT)$$

where n is the concentration of trapped electrons per unit volume at the time t. Eqn. (6) and (7) combine to give

$$i_l = \{1/(2l-1)\}\, n s_l \exp(-E_{al}/kT) \quad (18)$$

In this communication an attempt has been made to utilise these equations for the analysis of TL glow curve with an aim to evaluate the values of l, s_l, and E_{al}.

Eqn. (6) after integration yields into

$$n_0 = l \int_0^{\infty} i_l\, dt \quad (9)$$

and

$$n = l \int_t^{\infty} i_l\, dt \quad (10)$$

If the system is heated following a linear heating rate b according to the relation

$$T = T_0 + bt \quad (11)$$

Eqn. (10) changes as

$$n = (l/b) \int_T^{\infty} i(T')\, dT' = l\, A_T \quad (12)$$

where A_T represents the area of the TL glow curve enclosed within the temperature range T to ∞ such that

$$A_T = (1/b) \int_T^{\infty} i(T')\, dT' \quad (13)$$

Further, eqn. (9) can be represented as

$$n_0 = (l/b) \int_{T_0}^{\infty} i(T')\, dT' = l\, A_0 \quad (14)$$

where

$$A_0 = (1/b) \int_{T_0}^{\infty} i(T')\, dT' \quad (15)$$

A_0 represents the total area enclosed within the TL glow curve. Rearrangement of eqn. (6), (7) and (12) gives

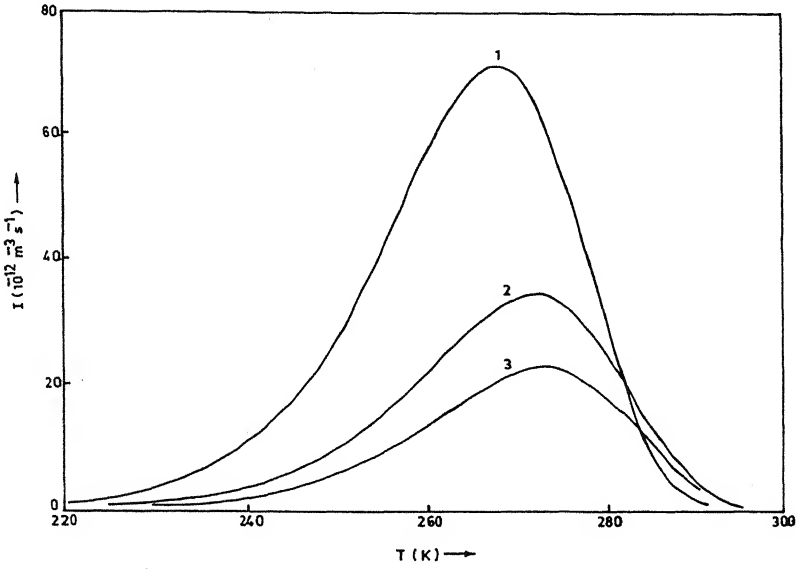

Fig. 1 – TL glow curves of different order of kinetics in a hypothetical system with $s_l = 2\times10^8\ s^{-1}$, $E_{al} = 0.55$ eV, b=0.1 Ks^{-1} and $n_0 = 2\times10^{16}\ m^{-3}$. Values of s_l and E_{al} are assumed to be the same for different order of kinetics. The number on the curves indicates the involved order of kinetics.

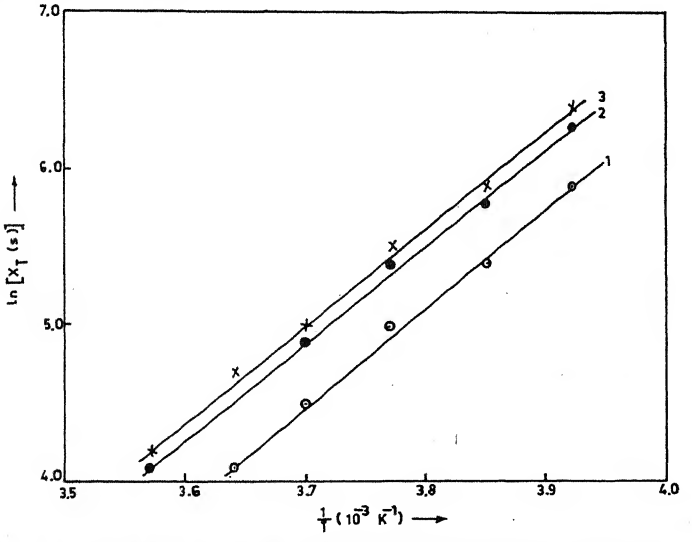

Fig. 2 – (Plot of $\ln(X_T)$ *vs.* $(1/T)$ for different order of kinetics in a hypothetical system recorded at $b = 0.1\ K\ s^{-1}$. The number on the straight lines indicates the involved order of kinetics.

Table 1 – Activation energy (E_{al}) and effective frequency factor (s_l) in a hypothetical system involving different order of kinetics with $s_l = 2 \times 10^8\ s^{-1}$ and $E_{al} = 0.55\ eV$.

Order of kinetics involved	Slope and intercept as per eqn. (19)			s_l (x $10^8\ s^{-1}$)
	E_{al} eV	$[(2l-1)/ls_l]$ ns	$[ls_l/(2l-1)] = s_l'$ (x $10^8\ s^{-1}$)	
1	0.55	4.97	2.01	2.0
2	0.56	7.56	1.32	2.0
3	0.55	8.44	1.18	2.0

$$X_T = \{(2l-1)/ls_l\} \exp(E_{al}/kT)$$

where $X_T = (A_T/i_l)$ (17)

In eqn. (17), i_l represents the intensity of TL glow curve of lth order of kinetics at the temperature T. Eqn. (16) can further be written as

$$\ln(X_T) = \ln[(2l-1)/ls_l] + (E_{al}/kT) \quad (18)$$

For a given TL glow curve, l, s_l and E_{al} are constant, so the plot of $\ln(X_T)$ *vs.* $(1/T)$ will be a straight line with the slope (E_{al}/k) and intercept equal to $\ln[(2l-1)/ls_l]$. Thus, the activation energy can be evaluated from the slope of the straight line plot. The intercept gives the value of $[(2l-1)/ls_l]$.

The value of n_0 can be known through some other independent experiment whereas A_0 is evaluated from the total area of the TL glow curve following eqn. (15). Knowing the values of A_0 and n_0, one can use eqn. (14) to know the value of l. This value of l helps in knowing s_l from the above mentioned intercept of the straight line plot. Thus TL decay parameters l, s_l and E_{al} can be determined.

TL glow curves for a hypothetical system involving different order of kinetics are shown in Fig. 1. Change in the shape of a TL glow curve in a hypothetical system with $E_{al} = 0.55\ eV$ and $s_l = 2 \times 10^8\ s^{-1}$ when it involves different order of kinetics can be visualised from Fig. 1. The evaluated values of X_T from Fig. 1 have been plotted as a function of $(1/T)$ in Fig.2. The slope and intercept determined as per eqn. (18) are presented in Table 1. It is obvious from the Table that in the case when $l=1$, the intercept of the straight line plot happens to be equal to s_l. Thus, the values of s_l and E_{al} can be evaluated when $l = 1$. However, in the cases when l is other than 1, the intercept gives the value of the effective frequency factor s_l' such that

$$s_l' = [ls_l/(2l-1)] \quad (19)$$

To evaluate the actual value of s_l in such cases it is thus necessary to know the value

of l. The value of l is determined from eqn. (14) provided n_0 is known through some other experiment. s_l represented in Table 1 is evaluated with the help of eqn. (19) knowing the value of l. TL glow curve can thus be analysed for evaluating the values of l, s_l and E_{al}.

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The decrease in relaxation time of nucleation of water nucleus due to presence of electric field

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Abstract The reduced relaxation time (ι_{on}), time taken for the formation of critical nucleus of water droplet or ice particle, has been found to decrease with increase in the supersaturation ratio. The decrease in ι_{on} suggests that the critical size of nuclei of water are formed earlier and hence more rains are possible in presence of external electric field provided sufficient water vapours are present in humid atmosphere. The effect of ions on the relaxation time has also been studied, the presence of ions, further indicated the decrease in relaxation time. This establishes the solar terrestrial relationship.

(**Keywords** : supersaturation ratio/lightning stroke/thunder cloud/ice glaciation/relaxation time.)

Murino[1] observed the effect of the electric field on condensation of water vapors and found that under similar conditions of temperature, a bigger size of droplet can be produced in smaller time in presence of electric field rather than in its absence. Sharma and Singh[2] compared the electric field with the supersaturation ratio. The calculations show that a small value of electric field is equivalent to very high supersaturation ratio to get a nucleus of given size under similar conditions of temperature. Moore *et al*[3] and Levin and Ziv[4] reported a rain gush following the lightning stroke. During a lightning discharge maximum electric field (~10 e.s.u.) is produced near the channel.

The inductive and non-inductive charging mechanisms of precipitation are efficient for thunder cloud electrification[5,6]. However, Vonnegut[7] and Moore[8] have shown that the convective charging mechanism is also responsible for thunder cloud electrification. On the other hand Sapkota and Varshneya[9] proposed an entrainment mechanism. The electric field growth in thunderstorm has been tested by using a parallel plate capacitor model[10]. It was found that the results of laboratory charge transfer experiments of ice crystal hailstone non-inductive mechanism can explain the observed electric field growth. Marshall *et al.*[11] explained the electric field growth and lightning initiate in thunderstorms. The lightning may limit the electric field inside the storm to values less than the

breakeven field, except for a brief interval just before each flash.

Singh *et al.*[12] have shown that in the resultant effect on a droplet due to an external electric field and the field induced due to the central dipole, the rate of nucleation in water vapour condensation and ice glaciation is about 100 times more near break down for dry air, as compared to that in absence of electric field.

Stolzenburg *et al*[13] compared results from nearly 50 electric field soundings through convective regions of mesoscale convective systems, isolated supercells, and isolated New Mexican mountain storms. Marshall and Stolzenburg[14] inferred total charge density and precipitation charge density. The cloud charge density was often zero in lower positive charge regions, between upper positive charge region and the bottom to the main negative charge region, the total charge density changes polarity and tends to become increasingly negative with decrease in altitude.

In the present communication the relaxation time for nucleation of water vapour condensation have been compared between ion induced and ion free cases in presence of electric field.

Theoretical Consideration :

The Gibb's free energy for formation of water embryo is

$$\Delta G = -(4/3)\pi r^3 \Delta G_v + 4\pi r^2 \sigma \quad (1)$$

where r is radius of embryo; σ, the macroscopic free energy (surface tension); ΔG_v the Gibb's free energy per unit volume. For critical size of water nucleus,

$$\frac{\partial \Delta G}{\partial r} = 0 \quad (2)$$

which gives the critical radius r^* of nucleus. The number of water molecules in critical nucleus is given by

$$n^* = \left(\frac{r^*}{1.958 \times 10^{-8}} \right)^3 \quad (3)$$

and the relaxation time

$$\tau_0 = \frac{9\pi k T (n^*)^{2/3}}{\mu^2 \beta \sigma} \quad (4)$$

where k is the Boltzmann constant; T, the temperature; β, frequency of collision of single molecule per unit area.

In presence of external electric field the relaxation time is expressed as

$$\tau_N = \frac{r^{*2/3}(2\rho_W)}{\left[3\rho(9\alpha\lambda E^2/m)\right]^{1/2}}$$

where ρ_W is the density of water; ρ, the density of water vapour molecules; α, the polarisability; λ, the mean free path;

E, the electric field and m, the mass of water vapour molecule.

The relaxation time for the growth of a nucleus in presence of an electric field varies inversely with the applied electric field. Under the combined effect of an electric field and diffusion, the reduced relaxation time τ_{ON} is

$$\tau_{ON} = \frac{\tau_O \tau_N}{(\tau_O + \tau_N)}$$

The values of τ_{ON} have been found to decrease with increase in supersaturation ratio $S_{V,W}$ as shown below :

At $T = 273$ °K. $E = 5$ e.s.u., $\lambda = 10^{-5}$ cm, $\alpha = 5 \times 10^{-23}$ cm^3, $m = 3 \times 10^{-23}$ g, $\rho_W = 1$. $\rho = 10^{-5}$

S. No.	$S_{V,W}$	r^* (Å)	$\tau_{ON}(\mu S)$
1.	1.01	1715.00	19840.00
2.	1.05	227.50	1995.00
3.	2.00	16.01	39.44
4.	3.00	10.10	19.84

In presence of ions, the Gibb's free energy is modified as

$$\Delta G' = \Delta G + \frac{3(ze^2)}{5r'}$$

where r' is new radius z, atomic number and e, the electronic charge.

The corresponding relaxation time τ_N^1 is found to decrease under the similar conditions.

Conclusions :From above discussions it is concluded that the ions produced in atmosphere due to galactic cosmic rays, decrease the relaxation time considerably. Hence the precipitation is enhanced. This enhancement is over and above the effect due to electric field.

Thus, the maximum electric field less than breakeven electric field dominates in the thunderstorms electrification. Further, improvements are under study modifying the polarisability by considering the molecule polarisability.

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On pathos line graph of a tree

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Abstract In this communication, the concept of pathos line graph is introduced. Its study is concentrated only on trees. We present a characterization of those graphs whose pathos line graphs are planar, outerplanar, maximal outerplanar, non minimally non outerplanar and eulerian.

(**Keywords** : pathos/path number/pathos line graph/ pathos point/pathos length.)

The concept of pathos of a graph G was introduced by Harary[1] as a collection of minimum number of line disjoint open paths whose union is G. The path number of a graph G is the number of paths in a pathos.

Stanton[2] and Haray[3] have calculated the path number for certain classes of graphs like trees and complete graphs.

The path number of a tree T is equal to K, where $2K$ is the number of odd degree points of T. Also the end points of each path of any pathos of a tree are odd points is given by Gudagudi[4].

All undefined terminology will conform with that in Harary[5]. All graphs considered here are finite, undirected and without loops or multiplelines. **The pathos line graph** of a tree T denoted as $PL(T)$ is defined as the graph whose point set is the union of the set of lines and paths of pathos of T in which two points are adjacent if and only if the corresponding lines of T are adjacent and the line lies on the corresponding path P_i of pathos. Since the system of pathos for a tree is not unique, the corresponding pathos line graph is also not unique. In Fig.1, a tree T and its different pathos line graphs $PL(T)$ are shown.

The line degree of a line uv of a tree T is the sum of the degrees of u and v. The pathos length is the number of lines which lie on a particular path Pi of pathos in T. A pendant pathos is a path Pi of pathos having unit length which corresponds to a pendant line in T. A pathos point is a point in $PL(T)$ corresponding to the path Pi of pathos in T.

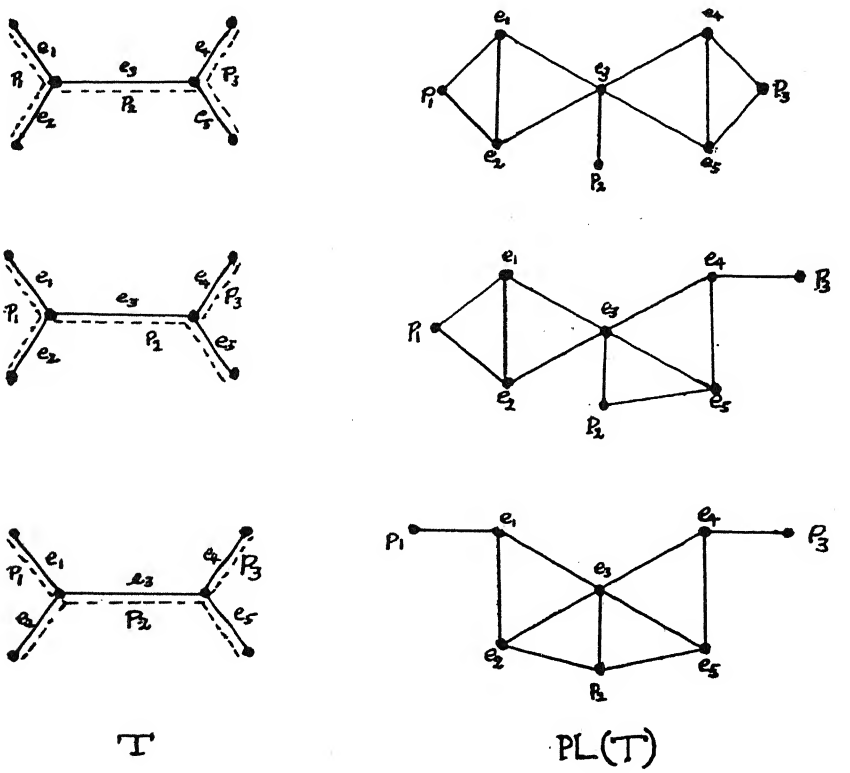

Fig. 1

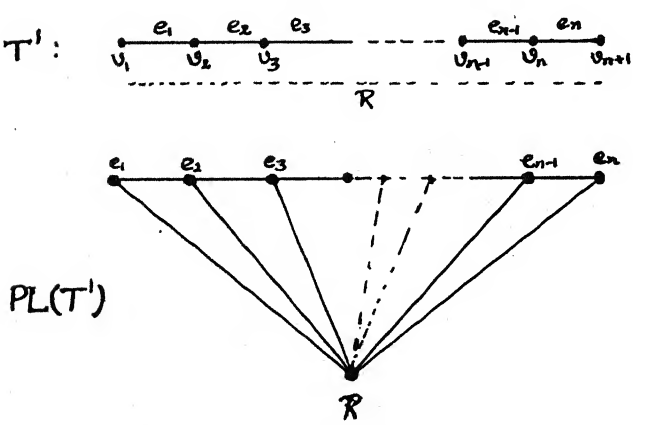

Fig. 2

If G is planar graph, the inner vertex number $i(G)$ of G is the minimum number of points not belonging to the boundary of the exterior region in any embedding of G in the plane. A graph is said to be minimally nonouterplanar if $i(G) = 1$ was given by Kulli[6].

We need the following results for our further results.

Theorem 1 [Ref. 5] : If G is a (p,q) graph whose points have degree d_i then $L(G)$ has q points and q_L lines where

$$q_L = -q + \tfrac{1}{2}\sum d_i^2$$

Theorem 2 [Ref. 5] : A graph G is a outerplanar if and only if it has no subgraph homeomorphic to K_4 or $K_{2,3}$.

Theorem 3 [Ref. 7] : A graph G is a nonempty path if and only if it is a connected graph with $p \geq 2$ points and

$$\sum_{i=1}^{P} d_i^2 - 4p + 6 = 0.$$

Theorem 4 [Ref. 8] : The line graph $L(G)$ of a graph G is planar if and only if G is planar, the degree of each point of G is atmost four and every point with degree four is a cutpoint.

Theorem 5 [Ref. 9] : The line graph $L(G)$ of a graph G is outerplanar if and only if the degree of each point of G is atmost three and every point of degree three is a cutpoint.

Theorem 6 [Ref. 5] : Every maximal outerplanar graph G with p points has $(2p - 3)$ lines.

Pathos Line Graphs

We start with a few preliminary results.

Remark 1 : The number of cutpoints in a line graph $L(T)$ is one less than the number of cutpoints in the tree T.

Remark 2 : The line degree of a line uv in a tree is odd if the degree of one point is even and the other odd.

Remark 3 : The line degree of every line in a tree is even if and only if every point is of odd degree.

Remark 4 : For any tree T, $L(T)$ is a subgraph of $PL(T)$.

Remark 5 : If a line in a tree T is of even line degree then the corresponding point in $PL(T)$ is of odd degree.

Remark 6 : If a line in a tree T is of odd line degree then the corresponding point in $PL(T)$ is of even degree.

Remark 7 : The degree of the pathos point in $PL(T)$ is equal to the pathos length of the corresponding path Pi of pathos in T.

Remark 8 : Every pendant pathos in a tree T corresponds to a pendantline in $PL(T)$ which adds one cutpoint to $PL(T)$.

In the following theorem we obtain the number of points and lines in $PL(G)$.

Theorem 7 : If a graph G is a (p,q) graph where points have degree d_i then its $PL(G)$ has $(q + k)$ points and

$$q_{PL(G)} = \tfrac{1}{2} \sum_{i=1}^{P} d_i^2 \text{ lines},$$

where k is the path number.

Proof : By definition of $PL(G)$, the number of points in $PL(G)$ is $(q + k)$.

By Theorem 1, the number of lines in $L(G)$ is $-q + \tfrac{1}{2} \Sigma d_i^2$. The number of lines in $PL(G)$ is the sum of lines in $L(G)$ and the number of lines which lie on the paths P_i of pathos of G which is q. Hence the number of lines in $PL(G)$

$$\text{is} = -q + \tfrac{1}{2} \sum_{i=1}^{P} d_i^2 + q,$$

$$= \tfrac{1}{2} \sum_{i=1}^{P} d_i^2.$$

Further we obtain a relation between line degree and degree of point in the following corollary.

Corollary 1 : For any line in a tree T with line degree n, the degree of the correspon-ding point in $PL(T)$ is $(n-1)$.

Proof : If a line in a tree T is of line degree n, then it is adjacent to $(n-2)$ lines. Since the line itself contributes one degree to each endpoint in T, $(n-2)$ degrees are contributed for the corresponding point in $PL(T)$. Also by definition of $PL(T)$, every line in T lies on only one path P_i of pathos. This contributes one more degree to the corresponding point in $PL(T)$. Hence the degree of the point in $PL(T)$ corresponding to a line in T with line degree n is $n-2+1 = n-1$.

Planar Pathos Line Graphs

A criterion for pathos line graph to be planar is presented in our next theorem.

Theorem 8 : The pathos line graph $PL(T)$ of a tree T is planar if and only if $\Delta(T) \leq 4$.

Proof : Suppose (T) is planar. Assume $\Delta(T) \geq 5$. If there exists a point v of degree 5 in T, then by Theorem 4, $L(T)$ is nonplanar. By Remark 4, $PL(T)$ is also nonplanar, a contradiction.

Conversely, suppose every point of T lies on atmost four lines. By Theorem 4, $L(T)$ is planar. Each block of $L(T)$ is either K_2 or K_3 or K_4. The lines joining these blocks from the pathos points are adjacent to atmost two points of each block of $L(T)$. This gives planar $PL(T)$.

We now present a characterization of trees whose pathos line graphs are outer-planar and maximal outerplanar.

Theorem 9 : The pathos line graph $PL(T)$ of a tree T is outerplanar if and only if $\Delta(T) \leq 3$.

Proof: Suppose $PL(T)$ is outerplanar. Assume that T has a point v of degree 4.

Then the lines incident to v form K_4 as an induced subgraph of $PL(T)$, a contradiction.

Conversely, suppose every point of T lies on atmost three lines. By Theorem 5, $L(T)$ is outerplanar. The lines joining to $L(T)$ from the corresponding pathos points gives $PL(T)$ in which each region is a triangle. By Theorem 2, $PL(T)$ is outerplanar.

Theorem 10 : The pathos line graph $PL(T)$ of a tree T is maximal outerplanar if and only if T is a path.

Proof : Suppose $PL(T)$ is maximal outerplanar. Then $PL(T)$ is connected. Hence T is connected. If $PL(T)$ is K_2 then obviously T is K_2.

Let T be any connected tree with $p \geq 2$ points, q, lines and having path number k. Then clearly $PL(T)$ has $(q + k)$ points

and $\frac{1}{2}\sum_{i=1}^{p} d_i^2$ lines. Since $PL(T)$ is maximal outerplanar, by Theorem 6 it has $[2(q + k-3)]$ lines.

Hence $\frac{1}{2}\sum_{i=1}^{p} d_i^2 = 2(q + k) - 3.$

$$=2(p-1+k)-3,$$

$$\sum_{i=1}^{p} d_i^2 = 4p + 4k - 10.$$

It is known that for a tree which is a path, path number k=1,

Thus,

$$\sum_{i=1}^{p} d_i^2 = 4p + 4(1) - 10 = 4p - 6.$$

$$\sum_{i=1}^{p} d_i^2 = 4p + 6 = 0.$$

By Theorem 3, if follows that G is a non empty path. Necessity is thus proved.

For sufficiency, suppose T is a path. We consider two cases.

Case 1 : Suppose T is K_2. Then $PL(T)$ is K_2. Hence it is maximal outerplanar.

Case 2 : Suppose T is a non empty path. We prove that $PL(T)$ is maximal outerplanar by induction on the number of points (≥ 3) of T. It is easy to observe that the pathos line graph of a path P_3 is a triangle, which is maximal outerplanar.

As the inductive hypothesis, let the pathos line graph of a non empty path T with n points be maximal outerplanar. We now show that the pathos line graph of a path T^1 with $(n+1)$ points is maximal outerplanar. First we prove that it is outerplanar.

Let the points and line sequence of the path T^1 be $v_1e_1v_2e_2v_3.....v_{n-1}\ e_{n-1}\ v_n\ e_n\ v_{n+1}$. T^1 and $PL(T^1)$ are shown in Fig. 2 Without loss of generality, $T^1 - v_{n+1} = T$. By inductive hypothesis, $PL(T)$ is

maximal outerplanar. Now the point v_{n+1} is one point more in $PL(T^1)$ than in $PL(T)$. Also there are only two lines (e_{n-1}, e_n) and (e_n, R) more in $PL(T^1)$. Clearly, the induced subgraph on the points e_{n-1}, e_n, R is not K_4. Hence $PL(T^1)$ is outerplanar.

We now prove that $PL(T^1)$ is maximal outerplanar. Since $PL(T)$ is maximal outerplanar it has $(2n-3)$ lines. The outerplanar graph $PL(T^1)$ has $2n-3+2=2(n+1)-3$ lines. By Theorem 6, $PL(T^1)$ is maximal outerplanar.

The following theorem gives a non minimally nonouterplanar $PL(T)$.

Theorem 11 : For any tree T, $PL(T)$ is not minimally nonouterplanar.

Proof: We have the following cases :

Case 1 : If $\Delta(T) \leq 2$, then by Theorem 10, $PL(T)$ is maximal outerplanar.

Case 2 : If $\Delta(T) \geq 3$, we consider the following subcases of Case 2.

Subcase 2.1 : Suppose $\Delta(T) = 3$, then by Theorem 9, $PL(T)$ is outerplanar.

Subcase 2.2 : Suppose $\Delta(T) > 3$. Assume that T has a point v of degree 4. Then T has subgraph homeomorphic to $K_{1,4}$. Clearly $\langle K_4 \rangle \subset L(T)$. Now $K_{1,4}$ has two paths of pathos and each of these pathos point are adjacent to two points of $\langle K_4 \rangle$ of $L(T)$. On embedding $PL(T)$ in any plane, $i[PL(T)] \geq 2$. Hence $PL(T)$ is not mini-mally nonouterplanar.

In the next theorem, we deduce the number of cutpoints in $PL(T)$.

Theorem 12 : If T is a tree with m cutp-oints, n pendant pathos and k paths of pathos each of length $l_i > 2$, then the number of cutpoints in $PL(T)$ is

$$m-1+n-\sum_{i=1}^{K}(l_i-2).$$

Proof : Suppose T has no cutpoints, then $T = K_2$ and $PL(T) = K_2$.

If T has $m \geq 1$ cutpoints, we have the following cases.

Case 1 : For $m = 1$, $T = K_{1,p}$ $(p \geq 2)$. We have the following subcases of Case 1.

Subcase 1.1 : If p is even, then $n = 0$ and $l_i-2 = 0$, The number of cutpoints in $PL(T) = 0$.

Subcase 1.2 : If p is odd, then $n = 1$ and $l_i - 2 = 0$. By Remark 8, the number of cutpoints in $PL(T) = 1$.

Case 2 : Let $m>1$, with n pendant pathos. In $L(T)$ each block is a complete subgraph $\langle K_p \rangle$, $p \geq 2$. Consider the following subcases of Case 2.

Subcase 2.1 : If a $l_i = 2$, clearly the pathos point is adfacent to two points of $\langle K_p \rangle$ of $L(T)$ in $PL(T)$ and this does not increase the number of cutpoints. By Remark 1 and Remark 8, the number of cutpoints in $PL(T) = (m-1+n)$.

Subcase 2.2 : If $l_i > 2$. Since a path of pathos passes through $(li-1)$ points in T, the corresponding pathos point is adjacent to $(l_i-)-1 = l_i-2$ cutpoints of $L(T)$ in $PL(T)$. This reduces the number of cutpoints in $PL(T)$ by (l_i-2). Hence taking summation of all these k paths of pathos, we get the number of cutpoints

to be reduced in $PL(T)$ as $\sum_{i=1}^{k} (l_i-2)$. Thus,

from case 2, we get the number of cutpoints

in $PL(T)$ as $m-1+n-\sum_{i=1}^{k} (l_i-2)$.

The following corollary gives a condition for the pathos length to be even.

Corollary 2 : If every line of a tree T is of line degree odd then the pathos length of every path is even.

Proof : Suppose every line of a tree T is of line degree odd. Then by Remark 2, T contains alternate even and odd degree points. Since every path of pathos starts and ends at odd degree points, the path must pass through atleast one point of even degree. Thus the pathos length should be atleast two or multiples of two. Hence the pathos length of every path is even.

The converse need not be true.

In the next theorem, we characterize the eulerian $PL(T)$.

Theorem 13 : Let T be any tree with $q \geq 2$ points and the line degree of each line in T be odd. Then $PL(T)$ is eulerian.

Proof : Consider the case when each line in T is of line degree odd. Then by Coro-llary 2, each path of pathos is of even length, by Corollary 1 and Remark 7, the degree of each point in $PL(T)$ is even. Hence $PL(T)$ is eulerian.

The case when T has atleast one line of line degree even. Assume that every path of pathos is of even length or atleast one path of pathos is of odd length. Then by Corollary 1 and Remark 7, $PL(T)$ contains atleast one point of odd degree, Hence $PL(T)$ is not eulerian.

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Locally semi-consecutive edge labelings of graphs

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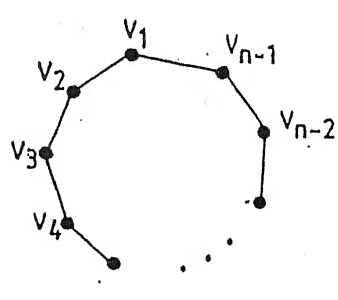
Abstract In this communication we introduce and study a new concept in the area of graph labeling problems *viz.*, the problem of determining graphs which admit an edge labeling called locally semi-consecutive edge labeling: A locally (semi)-consecutive (or *l*(*s*)c) edge labeling of a graph G is defined as an assignment of distinct positive integers to the edges of G so that at each vertex of G having degree at least two, all (or at least two) of their incident edges receive consecutive integers. A graph which admits an *lsc* (*lc*) edge labeling is called an *lsc* (*lc*)-graph. We prove that all trees are *lsc*-graphs and characterize both *lsc* and *lc*-graphs.

(**Kewwords** : locally consecutive edge/ locally semi-consecutive edge labelings)

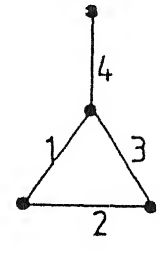
For all standard terminology and notations in graph theory we follow Harary[1]. Unless mentioned otherwise, all graphs considered in this communication are finite and simple.

An edge labeling of a graph $G = (V, E)$ is an injective function $g : E(G) \dashrightarrow \{1, 2, 3, \ldots\ldots, |E|\}$. Further, it is said to be locally (semi)-consecutive (or *l*(*s*)*c*), if for every vertex v with $d(v) \geq 2$, the set $g(E_v)$ where $E_v = \{e \in E : e = vu \text{ for some } u \in V\}$, consists of (contains at least two) consecutive integers. We then call a graph *l*(*s*)*c*-graph provided it admits an *l*(*s*)*c* edge labeling. Fig. 1 illustrates these notions.



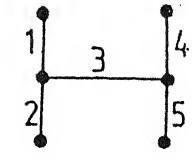
C_n : A non-lsc graph

(a)



An lsc graph

(b)



An lc graph

(c)

Fig. 1

A (one way infinite) caterpillar is a connected graph in which the set of vertices of degree exceeding one induce a (one way infinite) path. A graph is said to be locally finite if the degree of each vertex is finite.

Theorem 1 : A connected locally finite graph G is an *1c*-graph if and only if G is either a finite or a one way infinite locally finite caterpillar.

Proof : Let G have a locally consecutive edge labeling $g : E(G) \dashrightarrow \{1,2, \ldots, |E|\}$. Let v_1 be the vertex for which $g(E_{v_1}(G)) = \{1, 2, 3, \ldots, a_1\}$. Let $v_2, v_3, \ldots, v_{a_1+1}+1$ be the vertices adjacent to v_1. We claim that no two of them are adjacent and $d(v_i) = 1$ for each $i = 1, 2, \ldots a_1$. Suppose $v_r, v_s \in E(G)$ for $2 \leq r < s \leq a_1 + 1$. Then $r \in g(E_{a_r}(G))$, a contradiction to the local consecutiveness of g. This also implies that $d(v_1) = 1$ for each $i = 2, 3, \ldots a_1$.

Next, because of the local consecutiveness of the edge-labeling g we see that $g(E_{v_{a_1+1}}(G)) = \{a_1, a_1+1, \ldots, a_2\}$. Let $v_{a_1+2}, v_{a_1+3}, \ldots, v_{a_1+a_2-1}$ be the vertices adjacent to v_{a_1+1}. Again, we can show similarly as above that no two of these (new) vertices are adjacent in G as also $d(v_{a_1+j}) = 1$ for $j = 2, 3, \ldots, a_1 + a_2 - 2$, except possibly $v_{a_1+a_2-1}$. We can continue this procedure indefinitely until, of course, all the vertices would have been exhausted if the graph were finite.

The above procedure renders the graph G with the property that set of values of degree exceeding one induces a (possibly a one way infinite) path in G and hence G must be either a finite or a one way infinite locally finite caterpillar.

Conversely, if G is a finite or a one way infinite locally finite caterpillar, then it can be represented on the plane as a bipartite graph with a bipartition $\{A, B\}$ such that no two vertices within the set A or B are adjacent and also such that no two edges cross each other. Then label the edges of G with the natural numbers from the "top" of the plane representation of the caterpillars going down sequentially, possibly indefinitely, towards its "bottom". The resulting labeling of the edges of G is obviously a locally consecutive edge labeling of G.

Remark 1 : If G is a finite caterpillar then one may consider a graceful numbering f of the caterpillar which yields the locally consecutive edge labelings $g_f : \dashrightarrow \{1, 2, \ldots, q\}$, defined by $g_f(uv) = |f(u) - f(v)|, \forall\, uv \in E(G)$. (see Fig. 2)

Remark 1 raises the problem of determining those graceful graphs which admit graceful numberings that induce *1sc* edge labelings.

Next, we give an algorithmic proof to show that all trees are *1sc* graphs.

Theorem 2 : All trees are *1sc* graphs.

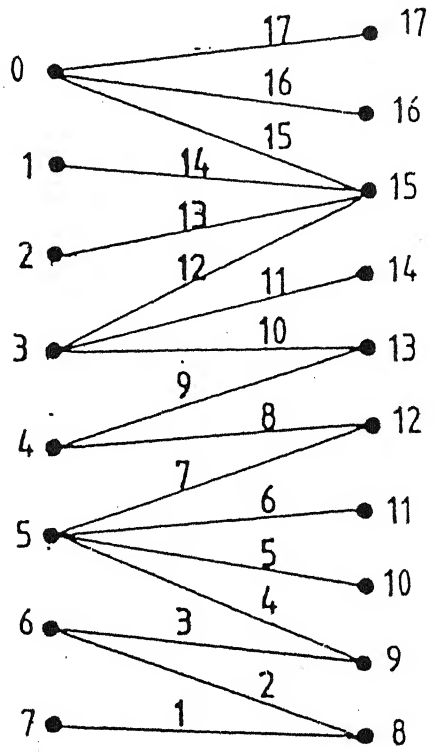


Fig. 2 – An *lc*-labeling from a graceful numbering of a finite caterpillar.

Proof: We shall give an algorithm to obtain an *lsc* edge labeling of a given tree T. Choose a longest path $P_t = (v_1, v_2, \ldots\ldots v_t)$ in T and define $g(v_i\, v_{i+1}) = i$, for $1 \le i \le t-1$. Note that v_1 and v_t are both pendant vertices in T. Next, consider the least integer i_o such that $2 \le i_o \le t-1$ and $d(v_{i_0}) \ge 3$. Let T_o be a branch of T rooted at v_{i_0}. We then choose a pendant vertex v_t+1 of T_o farthest from v_{i_0}, Consider the unique $v_{i_0}\, v_t+1$–path $P_1 = \{v_t+1, v_t+2, \ldots., v_{t+r}, v_{i_0}\}$ and label its edges successively starting from the edge $v_{t+1}\, v_{t+2}$ to $v_{t+r}\, v_{i_0}$ by numbers t, $t+1$, $\ldots\ldots t+r$ in that order. If there are no more branches in T_o rooted at any of the vertices on P_1 then we choose the smallest integer i_1, such that $i_o \le i_1 \le t-1$, and $d(v_{i_1}) \ge 3$ and repeat the foregoing process. On the other hand, if there are branches in T_o rooted at one of

the vertices of P_1 then we repeat the application of the above method of assigning labels to the edges of those branches, till we exhaust all such branches in T_o. The same procedure may be applied to label the edges of other branches rooted at the vertices on P_t, wherever they exist, in a sequential manner. Clearly, this procedure exhausts all the edges of T and also that at every vertex of T whose degree exceeds one, at least two edges receive consecutive integers.

Remark 2 : One can see that the procedure followed above is the Depth First Search (DFS) method (see Deo[2] for DFS).

An edge labeling of a tree obtained by applying the procedure described in the proof of Theorem 2 is exhibted in Fig. 3.

Theorem 3 : No 2-regular graph is an *Isc* graph.

Notice that if G is an *Isc* graph, then in every *Isc* labeling every vertex of degree two must have its incident edges labeled consecutively. This motivates the following result.

Theorem 4 : Suppose that a connected graph G has a maximal trail starting from a vertex of degree $\neq 2$ containing all the vertices of degree ≥ 3. Then G admits an *Isc* labeling.

Proof : Let G be a graph satisfying the hypothesis of the theorem and let $T = (e_1, e_2, \ldots, e_k)$ be a maximal trail containing all the vertices of degree ≥ 3. Then assign i to the edge e_i of T. Consider a vertex which does not lie on T. Such a vertex must have degree ≤ 2. Since G is connected any such vertex u in T must lie on a path or a cycle which has just one vertex common with T. In particular if u lies on such a cycle then the maximality of T implies that all the edges of the cycle must have been covered by T. Hence, we need to consider only the vertices which lie on a path having just one vertex of T,

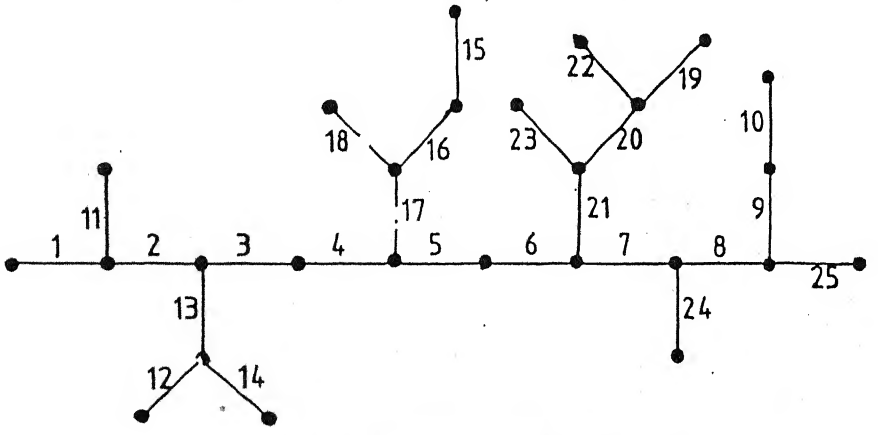


Fig. 3- An algorithm to *Isc* label of a given tree.

called its 'root'. Let the vertex sequence of T be $(u_o, u_1,.....,u_k)$. Let u_i be any vertex in this sequence at which enrooted paths are P_{i1}, $P_{i2},.....P_{in}$ each of whose edges are yet to be labeled. Let us call the edges of P_{ir}, $1 \leq r \leq n$, which is incident to u_i the "bottom edge" of P_{ir} and call the pendant edge of P_{ir} its "top edge". We then label the edges of P_{i1} by numbers $k+1$, $k+2,.....,k+t$ successively from the bottom edge to the top edge of P_{i1}. Hence, again we label the edges of P_{i2} by number $k+t+1$, $k+t+2,....., k+t+s$, successively from the bottom edge of P_{i2} to its top edge. We can thus exhaust labeling the edges of all the paths. P_{i1}, $P_{i2},.....,P_{ir}$ enrooted at u_i. We can continue the same process on the edges of the paths enrooted at any other vertex on T at which paths with unlabelled edges occur by assigning the number $m+1$, where m is the maximum of the already assigned labels. In this manner we can exhaust labeling the edges of all the paths enrooted at the vertices of T.

Since u_o has degree $\neq 2$, one sees that it is either 1 or at least 3. If $d(u_o) \geq 3$ we are through, by hypothesis. On the other hand, if $d(u_o) = 1$ we have nothing to prove. Similar argument holds for the vertex u_k of T.

Lastly, assign the number left out by the above procedure from the set $\{1, 2,.....,q\}$ to the remaining edges of G in a one–to–one manner.

One can then see easily that the assignment of numbers by the above procedure results in an *1sc* labeling of G.

Corollary 1: Any connected graph having exactly two vertices of odd degree is a *1sc* graph.

Proposition 1 : An eulerian graph G is a *1sc* graph if and only if at least one vertex of G has degree ≥ 4.

Proof : Sufficiency follows from Theorem 4 as G has an eulerian circuit.

For the converse, suppose G has an *1sc* labeling. Then since G is not a cycle there must be at least one vertex of degree $\neq 2$. But since G is eulerian, every vertex degree is even whence the result follows.

Proposition 2 : A Hamiltonian graph G is an *1sc* graph if and only if at least one of its vertices has degree ≥ 3.

Proof : Since G is Hamiltonian there exists a spanning cycle C_n in G. Hence, the existence of a vertex of degree ≥ 3 implies sufficiency, by Theorem 4.

Conversely, if g has an *1sc* labeling, it cannot be a cycle on its own right so that its Hamiltonianness implies necessity of the stated condition.

Though a large number of classes of graphs, apart from the ones covered by the above results, can be shown to be *1sc* by the application of Theorem 4, the condition stated in the theorem is not sufficient.

The following result characterises the *lsc* graphs.

Theorem 5 : A graph is an *lsc* graph if and only if no component of it is isomorphic to a cycle.

Proof : It is known (Theorem 10.5, Bondy and Murty[3]) that if G is a 2–edge connected graph, then there exist subgraphs B_i of G such that $B_o \subset B_1 \subset B_2 \subset$ .....$\subset B_k = G$ where B_0 is a cycle and B_{i+1} is an edge disjoint union of B_i and P_i where P_i is a path in which only the end vertices belong to B_i. This shows that any 2–edge connected graph other than a cycle has an *lsc* labeling. For the general graph, the result follows from this and by repeated application of Theorem 2.

Converse follows from Theorem 3.

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A unimodal sequence for hypergraphs

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Abstract A new property of a certain sequence associated with matchings in a hereditary hypergraph is exhibited.

(**Keywords** : hypergraphs/unimodal sequence)

For standard terminology and notation in hypergraph theory we refer to Berge[1]. New terms and notations shall, however, be specifically defined whenever necessary.

Let $H = (X, \mathcal{E})$ be any (finite) simple hypergraph. Then $\mathcal{F} \subseteq \mathcal{E}$ is called a matching if no two distinct edges in $\mathcal{F}$ share a vertex in common (cf. : Berge[2], p. 64) : *in particular, if* $\cup_{F \in \mathcal{F}} F = X$ in this definition then we shall call $\mathcal{F}$ a 1-factor of H. The maximum number of edges in any matching of H is called the matching number of H and is denoted $\beta_1(H) := m$ say. Matching number (also, known in graph theory as *edge-independence number;* e.g., see Harary[4]) of a hypergraph has been studied by many authors (e.g., see Berge[1,2] Bollobas *et al*[3]. In this communication, we exhibit a new property of a certain sequence associated with matchings in a hereditary hypergraph.

Let $a_i = a_i(H)$ denote the number of ways to select a matching consisting of i edges of H and $r = r(H)$ denote the least subscript such that a_r is maximum

Next, for any given positive integer t, H is called *t-hereditary (or,* just *hereditary* if $t = 1$) if

(1) $A \subseteq X$, $|A| \geq t$, $B \in \mathcal{E}$ and $A \subset B \Rightarrow A \in \mathcal{E}$

Remark 1 : *In any matching $\mathcal{F}$ of a hereditary hypergraph H every edge must be maximal (w.r.t. set-inclusion).*

Given a hypergraph $H = (X, \mathcal{E})$ and $x \in X$, we define its *vertex (x)-extinguished associate* $H\text{-}(x)$, as the hypergraph $(X\text{-}\{x\}, \mathcal{F}_x)$ where $\mathcal{F}_x = \{E\text{-}\{x\} : E \in \mathcal{E}\}$.

Theorem : For any hereditary hypergraph $H = (X, \mathcal{E})$ and any vertex $x \in X$.

(i) $a_0 < a_1 < a_2 < \ldots < a_r \geq a_{r+1} > \ldots\ldots > a_m$, and

(ii) $r(H) - r(H\text{-}(x)) \in \{0,1\}$.

Proof : We prove the result by induction on the number of vertices in H. When H has just one vertex, *(i)* is trivial and *(ii)* holds vacuously. Suppose that the theorem has already been verified for all hereditary hypergraphs with fewer than $|X|$ vertices. Select an arbitrary vertex x, and let $u_1, u_2 \ldots\ldots u_d$ be the vertices *adjacent to x (that is,* u_i and x belong to some edge of H for each i). Now, any matching of size i either has no edge incident with x (so it is also an i-subset of $\mathcal{E}(H\text{-}(x))$, or else due to the hereditary property of H it must consist of a maximal edge E containing both u_j and x together with edges in a matchng of size $(i\text{-}1)$ in $(H\text{-}(x)\text{-}(u_j) := H\text{-}(x)\text{-}(u_j)$. Consequently, for each i

(1) $a_i(H) = a_i(H\text{-}(x)) +$

$$\sum_{j=1}^{d} a_{i-1}(H-(x)-(u_j)).$$

Now, let $r = r(H\text{-}(x))$. By the inductive hypothesis, for each j, $r(H\text{-}(x)) - r(H\text{-}(x)\text{-}(u_j)) \in \{0,1\}$. Thus, for each $i < r$ and for each j we know by the induction hypothesis that $a_j(H\text{-}(x)) < a_{i+1}(H\text{-}(x))$ and $a_{i-1}(H\text{-}(x)\text{-}(u_j)) < a_i(H\text{-}(x)\text{-}(u_j))$. Summing these inequalities and then substituting from (1) one verifies that $a_i(H) < a_{i+1}(H)$ for $i < r$. Similarly, when $i > r$ we know that $a_i(H\text{-}(x)) > a_{i+1}(H\text{-}(x))$ and $a_{i-1}(H\text{-}(x)\text{-}(u_j)) \geq a_i(H\text{-}(x)\text{-}(u_j))$. Since at least one inequality is strict, we may sum and sustitute from (1) to obtain the strict inequality $a_i(H) > a_{i+1}(H)$ for $i > r$.

Note that this procedure does not allow us to draw any conclusion about $a_r(H)$ and $a_{r+1}(H)$, but either $a_r(H) < a_{r+1}(H)$ and so $r(H) = r(H\text{-}(x)) + 1$, or else, $a_r(H) \geq a_{r+1}(H)$ and $r(H) = r(H\text{-}(x))$. In either case, we have verified (i) and (ii) and hence the proof is complete.

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The (n,m)-total domination number of a graph

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Abstract A set T of vertices in a graph $G = (V,E)$ is an (n,m)-total dominating set of G if each vertex $v \in V$ is adjacent to at least n vertices in T and m vertices in $V - T$. The (n,m)-total domination number $\gamma_{tn,m}(G)$ of G is the minimum cardinality of an (n,m)-total dominating set. In this communication, we characterize (n,m)-total dominating sets which are minimal, obtain some bounds on $\gamma_{tn,m}(G)$ and its exact values for complete graphs, complete bipartite graphs, cycles and wheels are found. We obtain a sufficient condition on a γ_{tn}-set of G which is a $\gamma_{tn,m}$-set.

(Keywords : domination/total domination/ n-total domination/(n,m)-total domination).

Throughout this communication $G = (V,E)$ represents a graph with p vertices and q edges. For any definition and terminology not given below, we refer the reader to Harary[1].

A set $D \subseteq V$ is a dominating set of G if each vertex in $V - D$ is adjacent to some vertex in D. The domination number $\gamma(G)$ of G is the minimum cardinality of a dominating set. For details on $\gamma(G)$, see Cockayne and Hedetniemi[2].

A dominating set D of G is a total dominating set if the induced subgraph $\langle D \rangle$ has no isolates. The total domination $\gamma_t(G)$ of G is the minimum cardinality of a total dominating set[3].

These two concepts were generalized in Fink and Jacobson[4] and Kulli[5] respectively as follows :

A set $D \subseteq V$ is an n-dominating set of G if each vertex in $V - D$ is adjacent to at least n vertices in D. The n-domination number $\gamma_n(G)$ of G is the minimum cardinality of an n-dominating set.

A set $T \subseteq V$ is an n-total dominating set of G if each vertex $v \in V$ is adjacent to at least n vertices in T. The n-total domination number $\gamma_{tn}(G)$ of G is the minimum cardinality of an n-total dominating set.

A set $D \subseteq V$ is an (n,m)-total dominating set of G if each vertex in $V-D$ is adjacent to at least n vertices in D and each vertex in D is adjacent to at least m vertices in $V-D$. This concept was introduced by Kulli and Sigarkanti[6]. The purpose of this communication is to study the (n,m)-total domination number of a graph as follows :

A set $T \subseteq V$ is an (n,m)-total dominating set of G if each vertex $v \in V$ is adjacent to at least n vertices in T and m vertices in $V-T$. The (n,m)-total domination number $\gamma_{tn,m}(G)$ of G is the minimum cardinality of an (n,m)-total dominating set.

A γ_{tn}-set is a minimum n-total dominating set. Similarly, a $\gamma_{tn,m}$-set can be defined.

The following result is straightforward, hence we omit its proof.

Theorem 1 : For any graph G,

$$\gamma_{tn,m}(G) \geq \gamma_{tn}(G). \qquad (1)$$

Theorem 2 : If $\gamma_{tn,m}(G)$ exists, then $\gamma_{tn+m}(G)$ exists.

Proof : Let T be a $\gamma_{tn,m}(G)$-set of G. Then there exists a set $T' \subseteq V - T$ such that every vertex in V is adjacent to n vertices in T and m vertices in T'. This implies that $T \cup T'$ is an $(n+m)$-total dominating set of G.

Next we characterize (n,m)-total dominating sets of G which are minimal.

Theorem 3 : An (n,m)-total dominating set T of G is minimal if and only if for each vertex $v \in T$ there exists a vertex $u \in N(v)$ such that $|N(u) \cap T| = n$.

Proof : Suppose T is minimal. On the contrary, suppose there exists a vertex $v \in T$ such that v does not satisfy the given condition. Then it follows that $T-\{v\}$ is an (n,m)-total dominating set of G, a contradiction. Hence v satisfies the given condition.

Converse is obvious.

The diameter of a connected graph G is the maximum distance between any two vertices of G and is denoted by *diam(G).*

Now we obtain an upper bound on $\gamma_{tn,m}(G)$.

Theorem 4 : For any graph G,

$$\gamma_{tn,m}(G) \leq p - m - 1. \qquad (2)$$

Further if the bound is attained, then diam(G) $\leq$ 3.

Proof : Let T be a $\gamma_{tn,m}(G)$-set of G. Since $V-T$ is a m-total dominating set of G,

$|V-T| \geq m + 1$.

This proves (2).

Now we prove the second part.

Suppose the bound is attained. Then it follows that $|V-T| = m + 1$ and $\langle V - T \rangle$ is complete. We consider the following cases :

Case 1 : Let $u,v \in V-T$. Then $d(u,v)=1$.

Case 2 : Let $u \in T$ and $v \in V-T$. Then there exists a vertex $w \in V-T$ such that u is adjacent to w. Thus $d(u,v) \leq d(u,w) + d(w,v) \leq 2$.

Case 3 : Let $u,v \in T$. Then there exist two vertices $w,x \in V-T$ such that u is adjacent to w and v is adjacent to x. Thus $d(u,v) \leq d(u,w) + d(v,x) + d(w,x) \leq 3$.

Thus for all $u,v \in V$, $d(u,v) \leq 3$ and hence $diam(G) \leq 3$.

Converse of this is not true. For example, if $\Delta(G) = p - 1$, $p \geq 5$ and $\delta(G) \geq 3$, then $diam\ (G) \leq 2$ and $\gamma_{t1,1}(G) = 2 < p-2$.

To prove a lower bound on $\gamma_{tn,m}(G)$, we prove the following :

Theorem 5 : For any graph G,

$$\gamma_{tn,m}(G) \geq \gamma_t(G) + n - 1. \qquad (3)$$

Proof : Let T be a $\gamma_{tn,m}$-set of G. Then $T' = T - \{v_1, v_2, \ldots, v_{n-1}\}$ is a total dominating set of G. Thus,

$$\gamma_t(G) \leq |T'| \leq \gamma_{tn,m}(G) - n + 1.$$

Hence, (3) holds.

Corollary 5.1 : For any graph G,

$$\gamma_{tn,m}(G) \geq p - q + n - 1. \qquad (4)$$

Proof : (4) follows from (3) and the fact that

$$\gamma_t(G) \geq \gamma(G) \geq p - q.$$

Now we obtain that exact values of $\gamma_{tn,m}(G)$ for complete graphs, complete bipartite graphs, cycles and wheels. First we state known results from Kulli[5].

Proposition A : For any complete graph K_p with $2 \leq n < p$.

$$\gamma_{tn}(K_p) = n + 1.$$

Proposition B : For any complete bipartite graph K_{p_1,p_2} with $1 \leq n \leq p_1 \leq p_2$.

$$\gamma_{tn,m}(K_{p_1,p_2}) = 2n.$$

Proposition 6 : For any complete graph K_p with $n + m + 2 \leq p$,

$$\gamma_{tn,m}(K_p) = n + 1. \qquad (5)$$

Proof : Let T be a γ_{tn}-set of K_p. Then by Proposition A, $|T| = n+1$ Since $\delta(K_p) \geq n + m + 1$, each vertex $v \in V$ is adjacent to at least m vertices in $V - T$. Thus T is an (n,m)-total dominating set of K_p and hence (5) follows from (1).

Similarly, we can prove

Proposition 7 : For any complete bipartite graph K_{p_1,p_2} with $2 \leq m + n \leq p_1 \leq p_2$

$$\gamma_{tn,m}(K_{p_1,p_2}) = 2n. \qquad (6)$$

Proposition 8 : For any cycle C_p with $p \equiv 0\ (mod\ 4)$

$$\gamma_{t1,1}(C_p) = p/2. \qquad (7)$$

Proof : Let $C_p : v_1v_2 \ldots v_pv_1$ be a cycle on p vertices. Then $T = \{v_1, v_2, \ldots v_{p-3}, v_{p-2}\}$ is a γ_t-set of C_p. Since each vertex in V is adjacent to exactly one vertex in

T and $\delta(C_p)=2$, it is also adjacent to a vertex in $V-T$ and hence T is a (1,1)-total dominating set of C_p. Thus (7) follows from (1).

Since the proof of the following result is similar to that of proof of (7), we omit its proof.

Proposition 9 : For any wheel W_p,

$$\gamma_{t1,1}(W_p) = 2. \quad (8)$$

Now we obtain a sufficient condition on a γ_{tn}-set of G which is also a $\gamma_{tn,m}$-set.

Theorem 10 : If $\gamma_{tn}(G) \leq \delta(G) - m$, *then any γ_{tn}-set of G is a $\gamma_{tn,m}$-set.*

Proof : This follows from the fact that each vertex in V is adjacent to at least n vertices in T and m vertices in $V - T$ where T is a γ_{tn}-set. of G.

Theorem 11 : Let T be a $\gamma_{tn,m}$-set G. If there exists a set $S \subset T$ with $|S| = k$ such that for any two vertices $u, v \in S$, $d(u,v) \geq 3$, then

$$\gamma_{tn-1,\,m}(G) \leq \gamma_{tn,m}(G) - k. \quad (9)$$

Proof : Clearly S is independent and every vertex in $V - S$ is adjacent to at most one vertex in S. Thus every vertex in V is adjacent to $n - 1$ vertices in $T - S$ and m vertices in $V - T \cup S$ and hence $T - S$ is an (n–1, m)-total dominating set G. This proves (9).

Theorem 12 :If G is an $n + m$ regular graph, then,

$$p/(m+1) \leq \gamma_{tn,m}(G). \quad (10)$$

Proof : Let T be a $\gamma_{tn,m}$-set of G. Since each vertex in V is adjacent to exactly n vertices in T and m vertices in $V - T$, we have

$$\gamma_{tn,m}(G).m + \gamma_{tn,m}(G) \geq p.$$

Thus (10) holds.

Next we obtain another lower bound on $\gamma_{tn,m}(G)$.

Theorem 13 : For any graph G,

$$\gamma_{tn,m}(G) \geq p - (q/n) + (n(n+1) + m(m+1))/2. \quad (11)$$

Proof : Let T be a $\gamma_{tn,m}$-set of G. Then,

$$q \geq |V-T|.\, n + n(n+1)/2 + m(m+1)/2.$$

This proves (11).

Theorem 14 : For any (n,m)-total dominating set T of G either $\langle T \rangle$ or $\langle V - T \rangle$ is not a block in G.

Proof : If G is disconnected, then $\langle T \rangle$ and $\langle V - T \rangle$ are disconnected and hence the result. Suppose G is connected. On the contrary, suppose both $\langle T \rangle$ and $\langle V - T \rangle$ are blocks in G.

Then each vertex in V is a cut vertex of G, a contradiction, since G has at least two noncut vertices. Hence the result.

Since the complement of an (n,m)-total dominating set of G is an (m,n)-total dominating set, we have

Theorem 15 : For any graph G,

$$\gamma_{tn,m}(G) + \gamma_{tn,n}(G) \leq p. \quad (12)$$

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Sequential and magic labeling of a class of trees

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Abstract We prove that a class of tree called T_p – Trees are magic and simply sequential.

(Keywords : magic labeling/sequential labeling/ T_p – trees.)

An enormous body of literature[1] has grown around the subject "*graph labelings*" especially in the last thiry years or so.

A (p,q) – graph $G = (V,E)$ is said to be magic if there exists a bijection $f : V \cup E \rightarrow \{1,2,3,.....(p + q)\}$ such that for all edges uv of G, $f(u) + f(v) + f(uv)$ is constant[2].

Given a (p,q)-graph $G = (V,E)$ and positive integer k, G is said to be *k-sequential* if there exists a bijection $f : V \cup E \rightarrow \{k, k+1, k + 2,....., k + q + q-1\}$ such that $f(u) = |f(u)-f(v)|$ for all $uv \in E$. A graph G that is 1-sequential is called *simply sequential*[3,4].

Transformed trees (T_p-trees) : Let T be a tree and u_0v_0 be two adjacent vertices in T. Let there be two pendant vertices u, v in T such that the length of u_0-u path is equal to the length of v_0-v path. If the edge u_0v_0 is deleted from T and u, v are joined by an edge uv, then such transformation of T is called an elementary parallel transformation (or an *ept*). (Acharya[5]).

If by a sequence of *ept's* T can be reduced to a path then T is called a T_p– *tree (Transformed tree)* and any such sequence regarded as a composition of mappings (*ept's)* denoted by P, is called a *parallel transformation of* T. The "path", the image of T under P is denoted as $P(T)$.

A T_p – tree and a sequence of two *ept's* reducing it to a path are illustrated in Fig. 1.

Theorem 1: Every T_p– tree is magic.

Proof : Let T be a T_p – tree with p vertices. By the definition of a T_p –tree there exist a parallel transformation P of T such that for the path $P(T)$ we have,

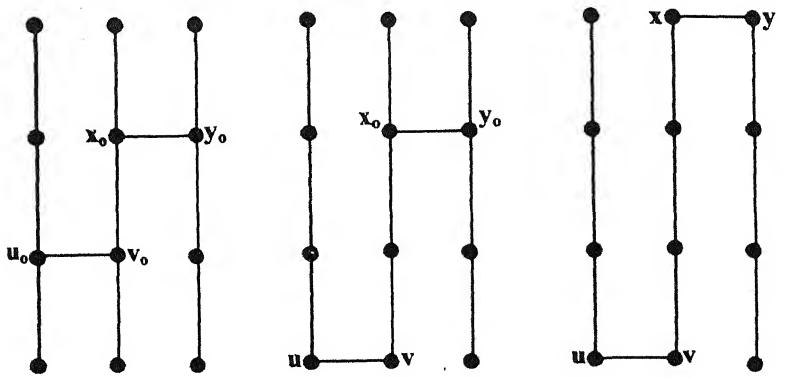

Fig. 1.

(i) $V(P(T)) = V(T)$

(ii) $E(P(T)) = (E(T) - E_d) \cup E_p$

where E_d is the set of edges deleted from T *and* E_p is the set of edges newly added through the sequence $P = (P_1, P_2, \ldots, P_k,)$ of the *ept's* P_i used to arrive at the path $P(T)$. Clearly E_d and E_p have the same number of edges.

Now label the vertices of $P(T)$ successively as $v_1, v_2, v_3, \ldots, v_p$ starting from one pendant vertex of $P(T)$ right up to other.

The labeling $f: V(P(T)) \cup E(P(T)) \rightarrow \{1,2,3,\ldots,p+q\}$ defined by

$$\begin{cases} f(v_i) = [4p-(1+i)]/2 & ; i \text{ odd}; 1 \le i \le p \\ f(v_i) = \begin{cases} [3p-(1+i)]/2 \text{ if } p \text{ is odd} & ; i \text{ even}; 2 \le i \le p-1 \\ [3p-i]/2 \text{ if } p \text{ is even} & ; i \text{ even}; 2 \le i \le p \end{cases} \\ f(v_iv_j) = (i+j-1)/2 & ; 1 \le i \le p-1, 2 \le j \le p \end{cases}$$

is clearly is bijective labeling of the path $P(T)$. It is enough if we prove the theorem for the T_p- tree with odd number of vertices (i.e. p is odd) because for even number of vertices (i.e. p is odd) because for even number of vertices the proof is analogous.

Let v_iv_j be any edge in $P(T)$, then $j = i + 1$.

If i is odd and $1 \le i \le p$ then,

$f(v_i) + f(v_j) + f(v_iv_j) = [4p-(1+i)/2 + [3p-(1+j)]/2 + (i+j-1)/2$

$= [4p-(1+i)]/2 + [3p-(1+i+1)]/2 + (i+i+1-1)/2$

$= (7p-3)/2$ (1)

If i is even and $2 \le i \le p$-1

$f(v_i) + f(v_j) + f(v_iv_j) = [3p-(1+i)/2 + [4p-(1 + j)]/2 + (i+j-1)/2$

$= (7p-3)/2$ (2)

From (1) and (2), for all edges v_iv_i be an edge in T for some indices i and j, $1 \le i \le p$ and let P_1 be the *ept* that deletes this edge and adge and adds the edge $v_i+_tv_{j-t}$ where "t" is the distance of v_i from v_{i+t} as also the distance of v_j from v_{j-t}. Let P be a papallel transformation of T that contains P_i as one of the constituent *ept*'s. Since $v_i+_tv_{j-t}$ is an edge in the path $P(T)$ it follows that $i+t+1 = j-t \Rightarrow j = i + 2t+1$. Therefore i and j *are of opposite parities.*

Now $f(v_{i+t}) + f(v_{j-t}) + f(v_{i+t}v_{j-t})$

$= f(v_{i+t})+f(v_{i+t+1})+f(v_{i+t}v_{j+t+1}))$

It $i + t$ is odd, then

$f(v_{i+t}) + f(v_{i+t+1}) + f(v_{i+t}v_{i+t+1})$

$= \{[4p-(1+i+t)]/2\} + \{[3p-(1+i+t+1)]/2\} + \{[(i+t+i+t+1-1)/2\}$

$= \{[4p-(1+i+t) + 3p-(2+i+t)+2(i+t)]/2$

$= [7p-3]/2$ (4)

If $i+t$ is even, then

$f(v_{i+t}) + f(f_{i+t+1}) + f(v_{i+t}v_{i+t+1})$

$= \{[3p-(1+i+t)]/2\}+\{[4p-(1+i+t+1)]/2\} +(i+t)$

$= [7p-3]/2$ (5)

$\therefore f(v_i) + f(v_j) + f(v_iv_j) = f(v_{i+t}) + f(v_{j-t}) + f(v_{i+t}v_{j+t})$

$= [7p-3]/2$

Hence T is magic

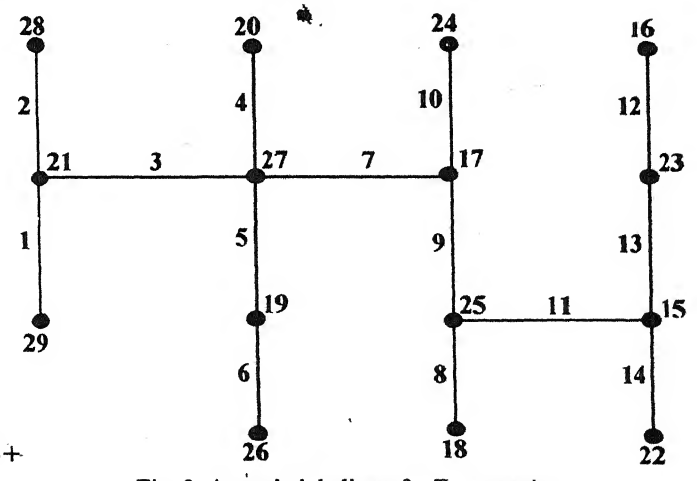

Fig. 2–A magic labeling of a T_p-tree using theorem 1.

Theorem 2 : Every T_p–tree is simply sequential. :

Proof : Let T be a T_p –tree with 'p' vertices. Then using elementary parallel transformation we get $P(T)$.

Then define

$f : V(P(T)) \cup E(P(T)) \rightarrow \{1,2,.., p+q\}$ by

$$f(v_i) = \begin{cases} 4p-(1+i)/2 & ; i \text{ odd } 1 \le i \le p \\ [2p+(i-2)/2 & ; i \text{ even } 2 \le i \le p \end{cases}$$

One can easily verify that f is a simply sequential labeling of $P(T)$. Let v_iv_j be an edge in T for some i,j, $1 \le i \le p$ and $v_i+_tv_{j-t}$ is the added egde for the deletion of v_iv_j by an *ept*, it follows from theorem 1 that $j=i+2t+1$.

Now $f(v_iv_j) = |f(v_i) - f(v_j)|$

$= |f(v_i) - f(v_{i+2t+1})|$

If i is odd and $1 \le i \le p-1$, then

$f(v_i) - f(v_{i+2t+1}) = \{[4p + (1+i)]/2\} - \{[2p + (i+2t+1-2)]/2\}$

$$= p-(i+t) \qquad (7)$$

$f(v_i) - f(v_{i+2t+1}) = \{[2p+(i+2)]/2\} - \{[4p - (1+i+2t+1)]/2\}$

$$= (i+t)-p \qquad (8)$$

From (6), (7) and (8) $f(v_iv_j) = |p-(i+t)|$ (9)

Now $f(v_{i+t}v_{j-t}) = |f(v_{i+t}) - f(v_{j-t})|$

$= |f(v_{i+t}) - f(v_{i+t+1})|$ (10)

If $i+t$ is odd, then

$f(v_{i+t}) - f(v_{i+t+1}) = \{[4p-(1+i+t)]/2\} - \{[2p + (i+t+1-2)]/2\}$

$$= p-(i+t) \qquad (11)$$

If $i+t$ is even, then

$$f(v_{i+t}) - f(v_{i+t+1}) = (i+t)-p \qquad (12)$$

From (9), (10), (11) and (12)

$f(v_iv_j) = f(v_{i+t}v_{j-t})$

Hence f is a simply sequential labeling of T_p– tree T.

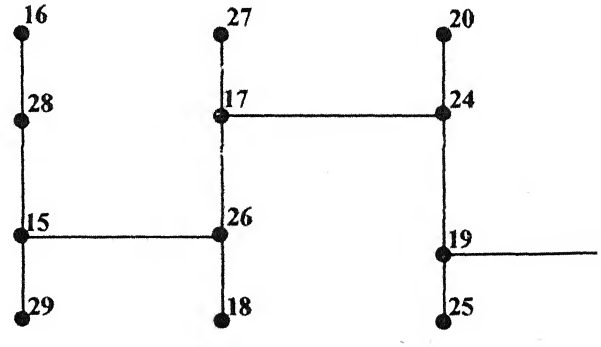

Fig. 3–A simply sequential labeling of a T_p-tree using theorem 2.

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Temporal functioning of enzyme systems in morpho-differentiation and growth of *Achatina fulica* (Ferussac).

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Abstract The activities of enzymes in the morphodifferentiation and growth of *Achatina fulica* revealed temporal changes. The activities of total phosphorylase reflect glycogen synthesis for the development and differentiation as well as for the oogenesis. SDH activity in digestive gland and body mass muscle indicates the involvement of these tissues in energy derivation. The differential activity of MDH reflect on the tissue level concentration of NAD and NADH and their flux. The lowered LDH activity in all the tissues suggest that the aerobic metabolism preponderates during development and morphodifferentiation. The activity of ATPase also concur with the above aerobic metabolism during development.

(**Keywords:** *Achatina fulica*/aerobic metabolism/ phosphorylase/oxidative enzymes)

Studies on enzymes are of interest as they initiate and catalyse several bio-chemical reactions leading to morphodiff-erentiation and development. In insects, studies on enzymes have revealed that the pattern of enzyme activity bear a direct correlation to the specific stages of dev-elopment[1-5].

However, such studies related to development in molluscs are meagre, though both the taxa share anatomical and physiological homologies. The giant African snail *Achatina fulica* has been established as a serious pest showing endemicity. Its pestiferous nature is evident from the damage it incurs to a wide variety of crops to the extent of limiting cultivation in some of the coastal and continental areas of India and also by its continuous dispersal to new areas[6]. In recent years, terrestrial snails have also been considered as an agricultural pests, as the humid agroclimatic conditions seems to favour the dispersal from their endemic areas to new areas. In India, the pest status of certain snail species has been recognized in view of their potential damage to several agricultural and horticultural crops[7,8].

In the temperate pulmonate, land snails which undergo hibernation and in case of temperate regions during dry season, the glycogen synthesis in different tissues attains importance as it is transfor-med into galactogen and reaches the eggs from albumin gland, before egg laying. The same mechanism also seems to operate in the snails belonging to tropical regions[9,10,11]. When snails are subjected to toxic stress, this mediates energy demand and substrate pressure and the changes in the glycogen content would be of interest

as its utilization could indirectly affect the eggs development.

Phosphorylase is responsible for the utilization of glycogen. Conformationally, it exists in two forms – tetrameric phosphorylase 'a' and phosphorylase 'b'. Both the phosphorylase 'a' and 'b' can adopt a catalytically inactive conformation or an active conformation[12]. The active phosphorylase brings about the degradation of glycogen. The estimation of phosphorylase activity could therefore, reveal about the availability of free sugars for energy derivation during stress. Its activity may also indicate the importance of glycogen in the development of *A. fulica.*

Oxidative enzymes are involved in energy metabolism and the derivation of energy is an essential pre-requisite for growth. Though, molluscs do not have the sequence of metamorphic changes in development, comparable to insects, the transitional events from immature phase to maturity and subsequently to post mature spent phase[13] are of interest, for the understanding of the biochemical events. Thus, the activity of oxidative enzymes could delineate the significance of them in morphodifferentiation and development in molluscs.

In the present investigation, the phosphorlyase 'a', 'b' and 'ab' and the oxidative enzymes such as, lactate dehydrogenase (LDH), succinic dehydrogenase (SDH), malate dehydrogenase (MDH) and adenosine triphosphatase (ATPase) were estimated at different phases of development of *A. fulica.*

Specimens of *A. fulica* were collected from local fields and gardens during rainy months (September – December), and reared in laboratory in netted plastic troughs containing wet sand. Snails were fed *ad libitum,* with cabbage and carrot during acclimation. The residues of food and the excreta were removed daily from the tank, to avoid contamination. Fresh food was given every day.

For the study of developmental profile, the snails taken from stock, were categori-zed into three groups *viz* (1) immature (2) mature/reproductive and (3) spent/post – reproductive phase. The immature group was further divided into two categories *viz.* Immature I & Immature II on the basis of the presence or absence or absence of protein gland (albumin gland). These two categories correspond to the juveniles and young adult groups as defined by Wolda[13] and Tomiyama[14]. According to peristome lip thickness, the snails were classified into three groups by Tompa[15].

Six snails were taken from the developmental stages, before taking the tissues, the haemolymph was collected from the intact snails using a butterfly vein needle. After collecting the haemolymph, digestive gland, albumin gland, body mass muscle and foot muscle were removed. The determination of haemolymph and tissues total phosphorylase 'ab', phospho-

rylase 'a' and phophorlylase 'b' was estimated by Cori *et al*[16]. The enzyme activity is expressed as 1 μ moles of Pi formed/hr/mg protein. LDH activity in tissues and haemolymph was determined following the procedure adapted by the King[17]. The enzyme was expressed as μg/100 mg (wet tissue). MDH activity in tissues and haemolymph was determined by Aquilina and Farmsworth[18]. The method used to estimate SDH activity in tissues & haemolymph is after Nachlas *et al*.[19]. . The enzymes were expressed in MIU/minute/mg protein. Adenosine triphosphatase (ATPase) was determined following the procedure of Shiosoko *et al*[20]. The unit of enzyme is defined as that amount which would liberate 1μ moles of Pi under incubation. Significance of values between developmental stages was determined by analysis of variance (ANOVA) Sokal and Rohalf[21].

Phosphorylase 'a'

The activity of phosphorylase 'a' in the different tissues of *A. fulica* at three different developmental stages viz immature phase I and II, mature and spent phases, is given in Fig. 1A. In the develop-mental stages, phosphorylase 'a' activity in the albumin gland, body mass muscle and haemolymph increased significantly in the mature and spent phases compared to that of immature phases ($p > 0.0001$) ($p = 0.05$) ($p = 0.003$) and ($p = 0.021$) ($p > 0.05$). In the mature phase, an insignifi-cant increase was observed in the foot muscle, ($p = 0.291$) ($p < 0.05$). In the spent phase (0.017070 moles), a slight decrease was noticed from that of immature phase I and II ($p > 0.05$) ($p = 0.05$). In the digestive gland, phosphorylase 'a' activity decreased significantly in the immature phase II, mature and spent phases compared to that immature phase I ($p < 0.0001$) ($p = 0.05$).

Phosphorylase 'b'

The activity of phosphorylase 'b' in the different tissues of *A. fulica* at three different developmental stages immature phase I and II, mature and spent phases, is given in Fig. 1B. In the mature and spent phases, the activity of phosphorylase 'b' in the body mass muscle, foot muscle and haemolymph, showed an insignificant decrease from that of immature phases ($p = 0.700$), ($p = 0.213$) and ($p = 0.157$) ($p < 0.05$) ($p = 0.05$). The activity of phosphorylase 'b' in the digestive gland, showed significant increase in the immature phase II, mature and spent phases from that of immature I ($p = 0.017$) ($p < 0.05$). In case of phosphorylase 'b' activity in the albumin gland, an insignificant increase was noticed in the mature and spent phases compared to that of immature phase II ($p = 0.414$) ($p > 0.05$).

Phosphorylase 'ab'

The activity of phosphorylase 'ab' in the different tissue of *A. fulica* at three different developmental phase viz immature I and II, mature and spent phases, is

given in Fig. IC. In the developmental stages, activity in the digestive gland, foot muscle and haemolymph decreased insignificantly in the mature and spent phases compared to that of immature phases I and II ($p = 0.120$) and ($p = 0.228$) ($p > 0.05$). However the total activity in the digestive gland of spent phase increased significantly from that of immature phase I and II. ($p = 0.003$) ($p < 0.05$) ($p = 0.05$). The activity of phosphorylase 'ab' in the albumin gland, showed, a significant increase in the mature and spent phases compared to that of immature phase II ($p < 0.0001$) ($p = 0.05$). In the body mass muscle, the activity increased insignifi-cantly in the mature and spent phases when compared to that of immature phase I and II ($p = 0.657$) ($p > 0.05$) ($p = 0.05$).

Lactate dehydrogenase

The activity of LDH in the different tissues of *A. fulica* at three different developmental phases is given Fig. 2A. With regard to developmental stages, LDH in the digestive gland, albumin gland and foot muscle, showed a significant decrease ($P<0.0001$) ($p = 0.05$) in the immature phase II, mature and spent phases compared to that of immature stage I. In the body mass muscle and haemolymph, the activity increased significantly in the mature phase but decreased significantly ($p < 0.0001$) ($p = 0.05$) in the spent phase compared to that of immature phase I and II.

Malate dehydrogenase

The activity of MDH in the different tissues of *A. fulica* at three different developmental phases is given in Fig. 2B. In the developmental stages, the activity of MDH in the digestive gland, foot muscle and haemolymph, showed a slight decrease in the mature and spent phases compared to that immature phase I and II. The decrease was insignificant to immature phase I and II ($p = 0.601$), ($p = 0.095$) and ($p = 0.582$) ($p>0.05$). In the albumin gland compared to that immature phase II, a significant increase in the mature and spent phases was observed ($p = 0.015$) ($p < 0.05$). The activity of MDH in the body mass muscle, showed an insignificant increase in the immature phase II, mature and spent phases compared to that of immature phase I ($p = 0.168$) ($p > 0.05$).

Succinic dehydrogenase

SDH activity in the different tissue of *A. fulica* at three different developmental phases is given in Fig. 2C. In the developmental stages, SDH activity in the digestive gland and body mass muscle, increased significantly ($p = 0.002$) ($p < 0.05$) in the immature phase II, mature and spent phases, compared to that of immature stage I ($p < 0.05$). In the albumin gland, foot muscle and haemolymph, it decreased significantly ($p < 0.0001$) ($p = 0.05$) in the mature and spent phases, compared to that of immature stage II. In the immature phase I and II enzyme activity was nil.

Adenosine triphosphatase

The activity of ATPase in the different tissue of *A. fulica* at three different developmental stages is given in Fig. 2D. In the developmental stages, the activity of ATPase in digestive gland, albumin gland, body mass muscle and foot muscle, increased significantly in the mature and spent phases, as compared to that of immature phase I and II ($p < 0.0001$) ($p = 0.05$) ($p < 0.002$) ($p = 0.05$). The activity of ATPase in the haemolymph, a slight insignificant decrease was noticed compared to that of immature phase I and II ($p = 0.984$) ($p>0.05$). But the spent phase showed an insignificant increase, as compared to that of immature phase I and II ($p=0.984$) ($p>0.05$).

In the developmental stages, the activity of total phosphorylase in the digestive gland, foot muscle, and haemolymph decreased in both the mature and spent phases. The activity of total phosphorylase in the albumin gland and body mass muscle increased in both the mature and spent phases compared to that of immature phase.

The activity of phosphorylase 'a' in the digestive gland, albumin gland, body mass muscle and haemolymph increased in both the mature and spent phases compared to that of immature phases. In the foot muscle, phosphorylase 'a' activity increased in the mature phase but a slight decrease was noticed in the spent phase. The phosphorylase 'b' activity in the digestive gland and albumin gland increased in both the mature and spent phases but decreased in both mature and spent phases of body mass muscle, foot muscle and haemolymph.

The total phosphorylase activity decreasing in the digestive gland, foot muscle and haemolymphs during the mature phase concur with the result on glycogen which showed an increase in the immature phases reflecting its synthesis for the development and differentiation as well as for oogenesis in which glycogen derived galactogen represents the stored reserved of energy for the embryos. Unlike mammals which possess endocrine and neuronal signals to activate the tissue specific glycogen phosphorylases the regulatory cascades are lacking in molluscs[22,23]. However, the present study on *A. fulica* indicates that in all its tissues, the phosphorylase activity occurs in the forms viz a and b and both take the active phase to flux the carbon into the glycolytic pathway, in the different sequences of development. Regarding oxidative enzymes during development, the increased SDH activity in the digestive gland and body muscle indicates the involvement in energy metabolism as compared to other tissues viz., the haemolymph, albumin gland and foot muscle. The lowered level of SDH in albumin gland could be expected by virtue of its function in the mobilisation of egg reserves and differentiation. Likewise, the foot muscle showing low SDH could also be expected by

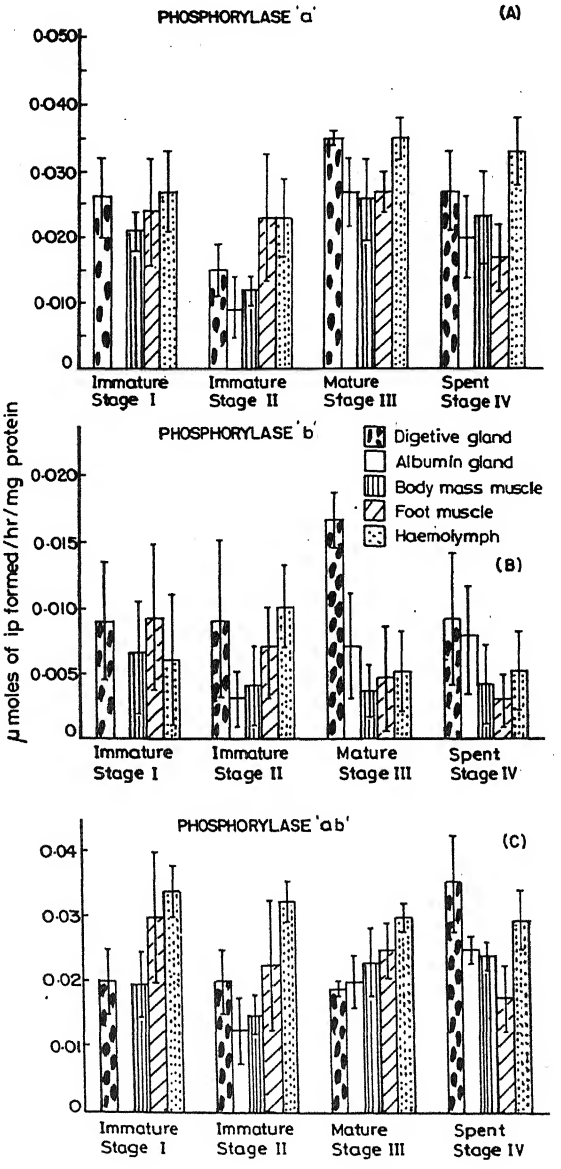

Fig 1 – (A) Phyosphorylase 'a'; (B) Phosphorylase 'b'; (C) Phosphorylase 'ab'. Phosphorylase activity in the different tissues of *A. fulica* in three different developmental stages viz., Immature stage I and Immature stage II; Mature or Reproductive stage and Spent or Post-reproductive stage ($p<0.05$ *Significant)

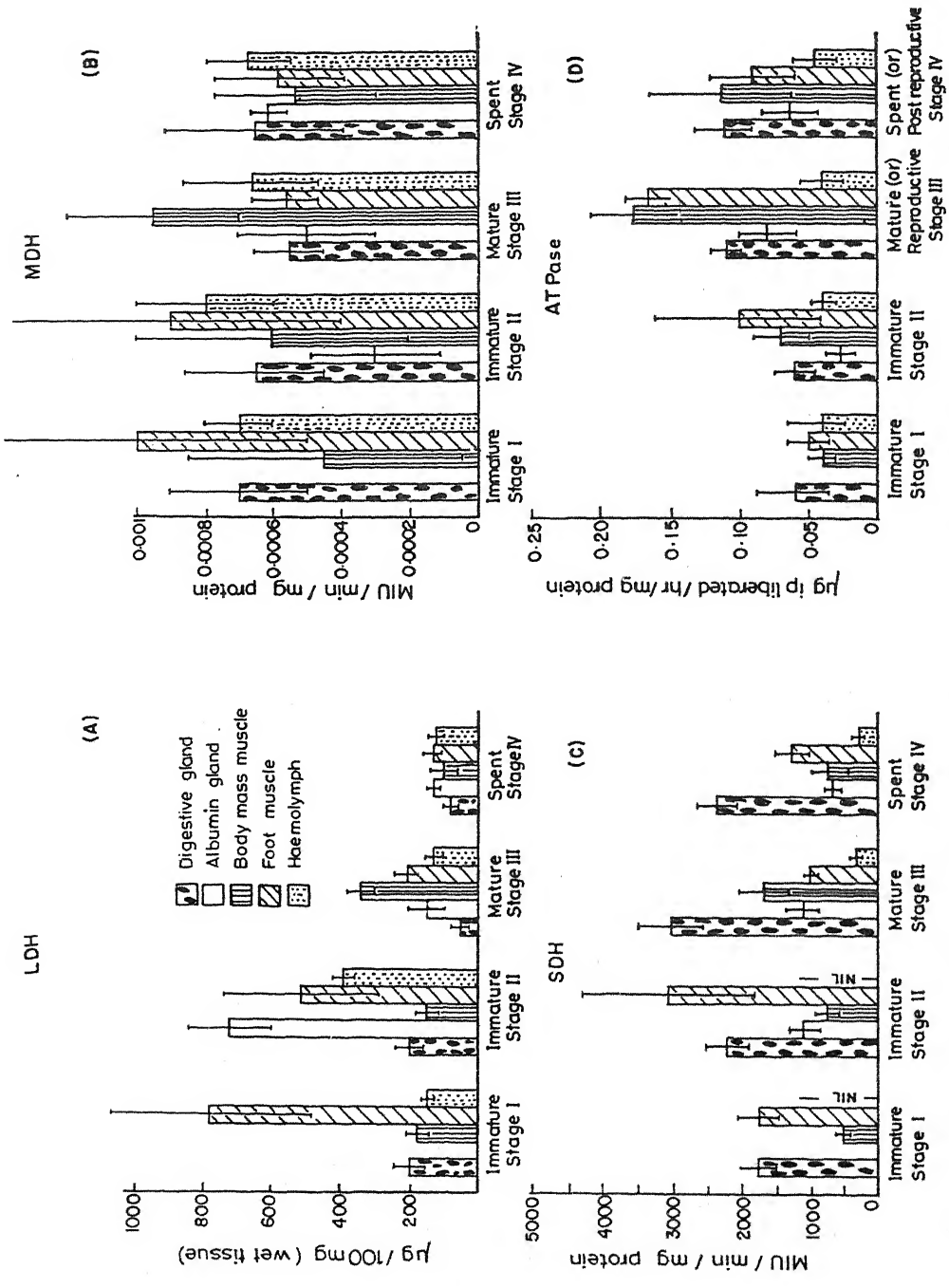

Fig. 2 – (A) LDH; (B) MDH; (C) SDH; (D) ATPase activity in the different tissues of *A. fulica* in three different developmental stages viz., Immature stage I and Immature stage II; Mature or Reproductive stage and Spent or Post-reproductive stage ($p<0.05$ *significant)

virtue of its anaerobic efficiency in function. Similarly, the differential activity of MDH in the various tissues also infers the tissue level concentration of NAD and NADH and oxaloacetate etc., to which the MDH show more affinity and function to bring about either the forward or reverse reaction. The lowered LDH activity in all the tissues during reproductive and post-reproductive phases also strengthens the suggestion that in normal development the aerobic metabolism only preponderates in the tissues.

In the development and differentiation, the hydrolysis of ATP by ATPase implies the energy requirement and its expenditure for several cellular functions such as active transport and reabsorption of essential elements etc. In the present study, ATPase activity in the disgestive gland, albumin gland, body mass muscle and foot muscle, increased in both the mature and spent phases as compared to immature phases I and II showing thereby the differential requirement of ATP formation and its utilization at different phases of development. As ATP and AMP concentrations *in vivo* also act as allosteric effectors for the functioning of enzymes like glycogen phosphorylase, isocitrate dehydrogenase (ICDH) and protein kinase, the above differences in ATPase function at different phases seem to be justified in the developmental study[24]. The results on the various categories of enzymes and their activities during the developmental phases of *A. fulica* reveal (i) each category of enzyme shows a differential activity in the different phases of development, (ii) the enzymes also differ in their activity in relation to the nature of organs/tissues and (iii) the above differential activity of the enzymes cultivate in totality the morphodifferentiation, growth, maturity and reproduction in these forms.

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Significance of nutritional gradients in the intestine of *Channa striata* in the niche selection by *Pallisentis nagpurensis*

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Abstract : The study of distribution of the different stages of an acanthocephalan parasite *Pallisentis nagpurensis* in the intestine of *Channa striata* revealed a selective preference of the small intestine (zone 3) by both sexes. Biochemical analysis of proteins, amino acids, glycogen, lipids and calcium revealed the existence of an anteroposterior gradient in the fish intestine. The prevalence of the low alkaline pH, abundance of low molecular weight nutrients and the favourable osmotic gradients in zone 3 are attributed as the preferential factors for the niche migration and selection by the parasite. Small intestine of *Channa striata* represents the zone of viability as well as fertility for the parasites. The parasites derive the nutrients both exogenously from enzyme digestion as well as endogenously through extra corporeal means from the intestinal wall of the host fish.

(**Keywords :** achanthocephala/*Channa striata*/ proximo-distal gradient/ osmotic gradient/ metabolites/niche selection.)

The distribution of different stages of the acanthocephalan parasite, *Pallisentis nagpurenis* (Bhale Rao 1931) differs with the region of the intestine of its final host *Channa striata*. The intestine was divided into five regions of which, region 4 and 5 viz., large intestine rarely contained the parasites. Fully grown up adults are restricted to regions 1 viz., duodenum of the intestine. The male parasites, which have copulated and the females, which carry early stages of achanthors are found in the region 2, the proximal part of the small intestine contiguous to duodenum. Region 3 viz., small intestine contained growing juveniles and those, which attained maturity. The region 3 of the intestine is termed not only a 'zone of viability' but also a 'zone of fertility', because mature parasites and those in copulated condition together with females carrying newly fertilized eggs are abundant in this region. Previous studies on the parasitic helminthes in vertebrate host suggest that the pH of the gastro-intestinal tract is an important factor for the uptake of metabolites by the parasites[1-3]. In addition, the proximo-distal gradient of metabolites in the gastro-intestinal tract also determines the growth, movement and development of parasites[4-7]. Thus there appears to be

a correlation between the movement and niche selection of the parasites and the biochemical composition of the worm. It also strongly suggests that the migration of the parasites is related to the nutritional and developmental requirements. However, information pertaining to the above aspects with reference to acanthocephalan parasites is scanty.

In the present study an attempt has been made to elucidate the biochemical ecology of the immediate environment of *P. nagpurensis* by studying the biochemistry of the fluid content at different regions of the intestine of the host. Such a study would not only enable us to understand the known postulated proximo-distal gradient of nutrients in the lumen of the intestine of the fishes but also enable correlation of the migratory movements of *P. nagpurensis* with changes in the chemical composition of luminal content of the host fish intestine.

Fishes (*Channa striata*) weighing 600-800 gms were analyzed soon after bringing to the laboratory. During transportation as well as in the laboratory, the fishes were kept without food. Thus the fishes were analyzed after 2 days of starvation as has been suggested by the previous workers[4,6,7]. Each fish was killed between 9 and 10 A.M. on the day when samples were required. Intestine was stretched out and the total length was measured in mms. It was divided into five sections and designated as regions 1,2,3,4 and 5 of the intestine. the average length of the fish intestine is about 130 mm and each section approximately measures about 26 mm in length. From 40 to 60% of the length was considered as region 3. Beyond this length the regions, have been designated as 4 and 5. After separating the different regions of the gut, each region was wrapped with Whatmann No. 1 filter paper to remove the body fluid. The luminal content of each region was squeezed out and it was collected in a test tube.

1 ml of luminal content was deproteinized with 3 ml of 80% ethanol and the precipitate was analyzed for protein content following Folin method of Lowry *et al*[8]. For determining total free sugars and free amino acids the ethanolic supernatants were used. Free sugar was determined following the method of Roe[9]. Total free amino acid content was estimated by the method of Frame *et al*[10] and the protein bound polysaccharide was determined by the method of Caroll *et al*[11]. Calcium was analyzed by chloranilic acid method of Webster's[12].

Prior to biochemical analyses, the total parasitic load in the different region of the intestine was calculated. The parasites collected from each region were then segregated into their respective stages following the morphological and morphometrical variabilities. The

number and the percentage of each stage, in relation to the total numbers were then calculated for both male and female. The total parasitic load and the number and percentage of each stage in both male and female *P. napurensis* in the five regions of the fish intestine were presented in Table 1.

regions. In region 1 it is 7.78 ± 0.82. There is no significant difference up to region 3, in which the pH is 7.68 ± 0.24. The pH becomes highly alkaline at region 4 (8.13 ± 0.59) and (8.34 ± 0.45).

The protein concentration of the intestine juice in general, ranges from 33 to 178 mg %. Interestingly, there exists

Table 1.– Distribution of different stages of growth of male and female *Pallisentis nagpurensis* in different regions of the intestine of *Channa striata.*

Growth Stages	Regions of the Intestine					Total
	1	2	3	4	5	
Male						
I	25 (2.5)	30 (3.0)	823 (81.4)	104 (10.3)	29 (2.9)	1011
II	19 (4.9)	103 (26.8)	245 (63.6)	13 (3.4)	5 (1.3)	181
III	126 (69.6)	9 (5.0)	43 (23.8)	2 (1.1)	1 (0.6)	
Total	170 (10.7)	142 (9.0)	1111 (70.4)	119 (2.1)	35 (2.2)	1577
Female						
I	18 (6.0)	18 6.0)	249 (82.7)	14 (4.7)	2 (0.7)	301
II	16 (7.8)	20 (9.7)	164 (79.9)	4 (1.9)	2 (0.9)	206
III	24 (11.8)	48 (23.5)	125 (61.3)	6 (2.9)	1 (0.5)	204
IV	43 (19.9)	153 (70.8)	19 (8.8)	0 (0.0)	1 (0.5)	216
V	296 (90.5)	5 (1.5)	24 (73)	2 (0.6)	0.(0.0)	327
Total	397 (31.7)	244 (19.5)	581 (46.3)	26 (2.1)	6 (0.5)	1254

Values mentioned in parenthesis refer to percentage of the total parasites distributed in the intestine.

Table 2 presents the values of various nutrients metabolites in the different region of the intestine. The pH of the intestine varies with different

a proximo-distal gradient in protein concentration in that the highest concentration being in region 1 and the lowest concentration being in region 5.

Table 2.– The values of biochemical constituents in the luminal content of the intestine of *Channa striata*.

No.	pH and Biochemical Parameters		Regions of the intestine				
			1	2	3	4	5
1.	pH	Mean	7.78	7.96	7.68	8.13	8.34
		±S.D.	0.82	0.7	0.24	0.59	0.45
				-----N.S.-----		-----P<0.02-----	
2.	Protein (mg%)	Mean	167.75	116.25	91.00	62.50	33.00
		±S.D.	60.62	47.49	51.01	34.48	20.74
				-----P<0.02-----		-----P<0.01-----	
3.	Protein bound polysaccharide (mg%)	Mean	19.52	8.72	15.02	7.33	5.70
		±S.D.	12.52	5.55	9.11	4.47	4.25
				-----P<0.01-----		-----P<0.01-----	
4.	Free sugars (mg%)	Mean	8.61	8.53	11.39	6.83	6.50
		±S.D.	7.02	6.33	8.01	6.55	5.10
				----- P<0.01-----		-----P<0.01-----	
5.	Free amino acid (mg%)	Mean	60.36	64.02	173.25	23.72	15.71
		±S.D.	20.18	37.00	65.35	11.39	8.64
				----- P<0.01-----		-----P<0.01-----	
6.	Calcium (mg%)	Mean	2.64	2.64	3.44	2.17	1.83
		±S.D.	1.18	1.18	1.27	1.17	1.09
				----- P<0.01-----		-----P<0.01-----	

N. S. : Not statistically significant with paired sample 't' test.

Sample size = 10.

The free amino acid concentration varies markedly in different regions of the intestine. The lowest free amino acid concentration is seen in region 4 and 5 (16 to 24 mg%). Almost thrice the amount of free amino acid is seen in region 1 and 2 (60 to 64 mg%). It is interesting to note that the region 3 has the highest amino acid concentration, as it has 173 mg%. Yet another noteworthy observation is that the variability observed in the free amino acid concentration of the fishes within a particular region as assessed by co-efficient of variation is lesser for free amino acids than for other biochemical components analyzed.

The protein bound polysaccharides also vary in relation to different regions. Significantly higher concentration of polysaccharides was observed in region 1 (19 mg%) and 3 (15 mg%). Free sugar concentration ranged between 7 to 9 mg % in region 1,2,4 and 5 and was significantly higher in region 3. The calcium content of the intestine ranged from 1.8 to 3.4 mg%. Region 3 contained highest calcium concentration.

The pH of the gastrointestinal tract of several vertebrates has been extensively investigated and frequently reviewed [1-2,13-16]. The importance of pH in the metabolites across the cell membranes, its relevance to *in vitro* experiments, and the interaction between the physiological mechanism of the alimentary canal of the host and those of a cestode living in the intestinal lumen have been appreciated since the early works of Kofoid[17] and Kofoid *et al*[13]. Information pertaining to fish intestine is however very meager. The pH of the intestine is considered to be neutral along the alimentary track. But, observations made in the present study reveal that the pH of the intestine of the *Channa striata* is alkaline. It varies with the regions of the intestine in that the pH is lowest in the region 3 highest in regions 4 and 5. It is already known that the parasitic load in the region 3 was maximum. The lowest alkaline pH is well matched with the high percentage of parasites. Similar results obtained by Mettrick[18-19] also revealed that the lowest alkaline pH of any region of the intestine was matched with the highest percentage of parasite biomass. Metrick[18] has attributed the lower intestinal pH of the parasitised intestine to the excretion of ions and other acidic end products of carbohydrate metabolism by *Hymenolepis diminuta*. The effect of reduced luminal pH on *H. diminuta* has been attributed to increase both fluid and glucose transport[20-21] and Na^+ ions transport[22]. The same may be true for *P. nagpurensis*. The high alkalinity may not be preferred by *P. nagpurensis* because the parasites are rarely found between region 4 and 5 wherein the pH ranges from 8.1 to 8.3.

The luminal content protein concentration showed distinct proximo-distal gradient as has been envisaged by Strobend and Van der Veen[4]. In all probabilities the protein analyzed in the lumen may be partly of endogenous origin secreted by the intestinal wall by the hydrolysis of dietary material as well as partly of exogenous origin delivered in these fishes. A number of authors have opined that the endogenous protein present in the intestinal lumen is quite sufficient to meet the protein requirements of helminthes[5-6]. However, besides the above endogenous sources from the host intestine, the dietary protein may represent an immediate source for uptake. The protein concentration is highest in region 1 and declines steadily

thereafter. Since the growing *P. nagpurensis* are restricted to region 3, wherein the protein is significantly lower than the previous regions, it is reasonable to infer that the nitrogen requirement of the growing of maturing parasites may be met by the large free amino acid pool occurring in this region. It is interesting to note that high concentration of free amino acids is not seen anywhere in the region of the intestine except for region 3. Moreover, the region may represent the active transport region of the absorbable metabolites viz., amino acids and glucose *in vivo*. Probably the lowest free amino acid concentration observed in region 4 and 5 may not favour the viability of the parasite in these regions. Similarly Mettrick[7] has shown that the large free amino acid pool occurring in the rat small intestine meets the nitrogen requirements of the growing cestode *H. diminuta*.

The proximal-distal gradient observed in the protein concentration of the luminal fluid may be one of the important biochemical factors responsible for attracting gravid females and males of stage III. It also envisages that the increasing gradient of the protein concentration in the anterior region acts as a factor in attracting the gravid female towards the anterior region. There is no doubt that after fertilization the energy demand of growing achantors may be on the increase. Probably the females after fertilization and males after copulation may have migrated to the regions of the better nourishment. As the region 4 and 5 highly alkaline and contained now protein and amino acid pools besides other components, the niche was shifted by migration to the proximal region 3. The parasites have found better precur-sors of their nutritional requirements in the later. Probably the permeability of the integument varies at different stages of development to facilitate sequestra-tion of proteins very similar to some of the free-living helminths[23]. It appears form this that the luminal protein gradient and accessibility of free amino acids may be a cause for anterior migration of the fully-grown adults.

Finally the results of this study corroborate with the findings of Mettrick and coworkers[24-28]. Observation of Cannon and Metrick[24] on the migration of *H. diminuta* within the rat intestine and the changes in the biochemical composition and metabolism of the worms associated with these migratory movements[25] suggest that the worms select appropriate but changing positions on one or more of the known or postulate nutritional gradients in the intestine. Similarly, the demonstration of the protein, amino acid and carbohydrates gradients in the intestine of rats and ducks[26-27] confirmed the existence of the nutritional gradients to which the parasites are probably

responding. In addition to biochemical gradients, which may possibly contribute differential localization of the parasites, Crompton[3] has stressed the need to consider several physical factors such as osmotic pressure on the localization and migration of the parasites.

In addition to protein nitrogen, the free sugar and protein bound polysaccharide and calcium are also abundant in region 3. Thus the region 3 represent a region of rich pool of low molecular weight nutrients. Probably because of this reason parasites of stages I, II and III of females and I and II of males select the above niche for deriving the above nutrients. As poikilothermic animals like fishes could retain the food content for long duration to enable complete digestion by the enzymes in the small intestine, the fish host may contribute both endogenous nutrients from their walls extra corporeally and enzymes hydrolyzed food constituents/nutrients of exogenous origin in vivo for the growth and development of the parasites.

The parasitic load in different regions of the intestine with reference to the different stages in both male and female and their percent variation conform well with the existing antero-posterior gradients of nutrients concentration. Thus, the contention that the nutrient gradients, availability of low molecular weight metabolites for easy uptake, and osmotic gradient of the content of the host, are the determining factors for the niche selection by *P. nagpurensis* seem to be tenable.

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Science News

The Poor Person Satellite—Solar Wings

The world's first solar–powered aircraft named helios successfully completed its 18 hours test flight over the pacific ocean this year. The unmanned aircraft of National Aeronautics and Space Administration (NASA) empowered by 62000 solar cells reached an altitude of almost 22 kilometers before descending. According to the official reports propulsion, avionics, environmental and flight control systems worked flawlessly during the flight.

The ultralight aircraft has a single, translucent, curved wing covered with 180 square metres of solar panels which were capable of generating 40 kilowatts of power. Only 10 kilowatts of energy is needed to keep the plane aloft. a couple of the pilots controlled the flight using desktop Computers. It attains a maximum of 300 km. per hour speed.

In researcher's view the aircraft is a "poor person satellite" and offers advantages over satellites in delivering telecommunication and television signals because of its low cost. Besides it can be easily grounded for repairs and payload changes.

Courtesy : Down to Earth

The Economy of Wetlands : Wetlands or Wastelands

In India 58.2 million hectares area of wetlands are regarded as wastelands despite the fact that they are beneficial to humans and are economically viable. Besides, they serve as reservoirs of ecological diversity. During floods they act as sponge by holding the water and releasing it slowly. Due to its large holding capacity it facilitates ground water recharging. Their excellent service to mankind is that they act as cleaners of pollutants which are discharged upstream. According to the recent researches the values of the wetlands are being appreciated in enumerate ways. The economic value of their services to human being is being evaluated. According to a recent report, published in 'Down to Earth'. value for recreation, value of existence and indirect values are 152.7, 432.9 and 219.2 million rupees per annum, respectively for Chilka lake in Orissa. However, the report further reterates that values of wetlands are invaluable in India as well as elsewhere.

Courtesy : Down to Earth

Plasma Membrane Channel Lisation of Calcium Ions — A Perspective Research in Cell Biology.

Calcium ions in general are regarded as important biological signals to certain regulatory processes within the cell. The physiological processes such as growth and development, protein secretion, muscle contraction and even cell deaths are signaled by involving an increase in the calcium ion concentration in the cell. One of the mechanisms by which this occurs is called 'capacitative calcium entry' which is also known as store – operated calcium entry. The process involves a regulated openning of ion channels in the outer cellular membrane i.e., plasma membrane. However, the molecular basis of these channels are yet to be established and unless and untill these molecules are identified our understanding regarding the valuable biological process will remain obscure. Our knowledge of the molecular and cellular mechanisms of capacitiative calcium ions may prove to be of enormous benefits to both, basic and applied researches.

The recent researches in the field were particularly devoted to two interrelated issues—

1. The identity of the capacitative calcium entry ion channels; and
2. The nature of the signal that activates them.

The solution of the first issue would constitute the basis for second one says James W. Puney. However, the electrophysiological experiments reveal that how the channels behave is known but their molecular components are still a mystery.

Courtesy : Nature

Pesticide Poisoning

'Phorate' pesticide has been listed in the US Environmental Protection Agency's list as a toxic element and only restricted use of the pesticide is allowed. However, the pesticide was being widely used in Kerala in Agricultural Practices. Lack of the information that the pesticide is a hazardous chemical resulted mortality of a 16 year old boy, a daily wage labourer in cardamum plantation. Same day 41 labourers were also brought to the notice of phorate poisoning. According to the reports the pesticide poisoning was a vexed question but the incident underlines the need of knowledge of the hazardous nature of chemicals used in agriculture.

Courtesy : Down to Earth

The Hardest Material

The search for a novel material with least compressibility and maximum hardness more or equal to diamond or

cubic boron nitride (Cubic BN), is on the global scale. In a recent report a group of martial scientists from Sweden, Australia and U.K. have reported to discover a cotunnite–structured titanium oxide which they claimed is the hardest known oxide so for and is basically a new polymorph of titanium dioxide, where titanium is ninecoordinated to oxygen in the cotunnite ($PbCl_2$) structure. The phase was synthesized at pressure above 60 gigapascals (Gpa) and temperatures above 1,000K.

Courtesy : *Nature*

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